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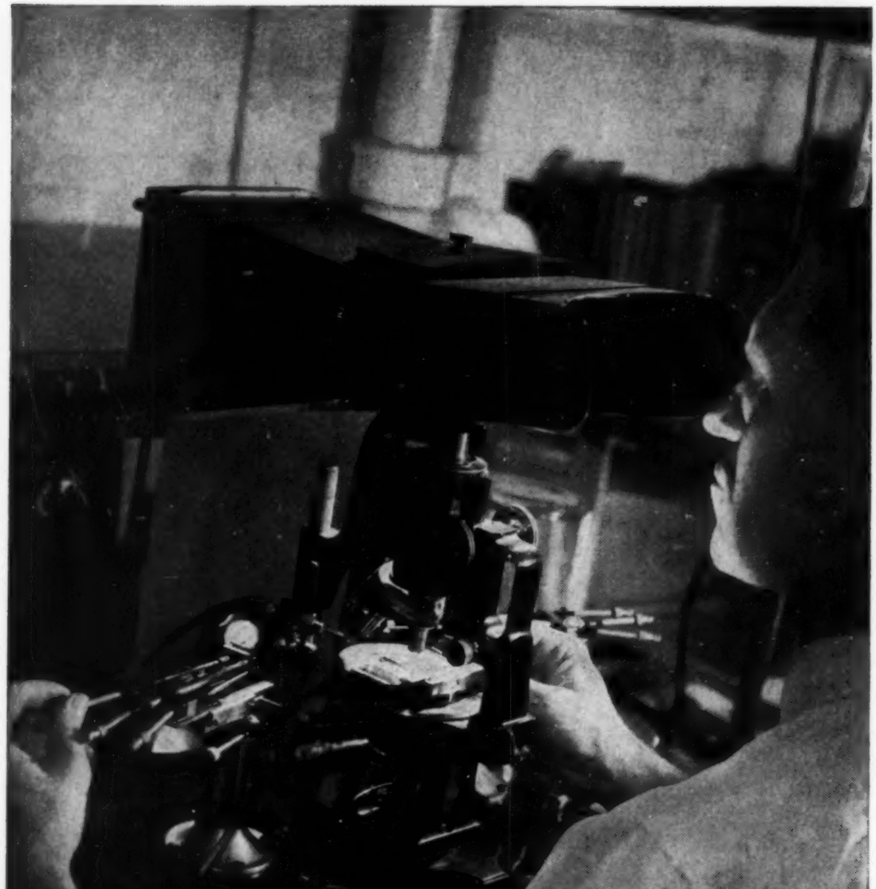

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EFFECTS ON FLOUR DOUGHS OF EXTRACTS FROM UNFERMENTED AND FERMENTED WHEAT GERM

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The effects on doughs of oxidizing substances, of reducing substances, and of proteases activated by —SH groups have for several years past attracted the attention of cereal chemists, and numerous investigations in this field have been carried out.

Shen and Geddes (1942) gave a comprehensive review of the literature dealing with these problems up to the second half of 1941. Their paper and other recent papers on the subject under discussion will be reviewed in the following paragraphs.

Shen and Geddes (1942) studied the effect of length of extraction and time of fermentation on bromated and nonbromated doughs and on doughs in which yeast activity was inhibited by octylalcohol. (This was added to prevent the yeast from using up the amino acids produced as a result of proteolysis.) They determined the amino nitrogen and reducing matter content of the doughs with a view to correlating them with the baking performance of the flours, and they reported that the fermentation time and/or bromate required to produce a satisfactory dough and loaf increased as the flours contained more of reducing matter and protease. On the other hand, however, they observed that the reducing matter content of the doughs increased with longer fermentation times, while it decreased when bromate was added. The amino nitrogen content of the nonfermenting dough increased with length of dough time.

Hullett (1940), investigating the effects on doughs of prefermented wheat germ suspensions, found that the nitroprusside reaction of a fermenting germ suspension becomes negative after a certain time, and he expressed the view that the elimination of glutathione may be an important part of the ordinary dough ripening process.

Hullett and Stern (1941), working on the same subject, showed that the elimination of glutathione from a fermenting germ suspension is

connected with the fermenting activity of the yeast on the one hand and with an enzyme mechanism present in the raw germ on the other. They reported that the disappearance of the nitroprusside reaction is not due to the oxidation of the —SH group of glutathione to the —S—S— form but to a more far-reaching change, and they showed that germ fermentation caused the nitroprusside reaction to become negative in the yeast as well as in the germ.

Smith and Geddes (1942) investigated the effect of 5% and 10% additions of germ, untreated and pretreated, on a highly refined untreated second middlings flour and on mixtures of this flour with 10%, 15%, or 20% of added wheat starch. The germ suspensions were pretreated by allowing them to stand for varying times with or without addition of bromate, or yeast, or yeast plus bromate. They found that the injurious effect of untreated wheat germ decreased as dough fermentation was extended from 1.5 to 4.5 hours. Addition of potassium bromate to the dough caused marked improvements of dough and loaf characteristics, whereas the addition of bromate to the aqueous germ suspension caused but little improvement. Aqueous germ suspensions, on standing, lost but little of their injurious effects, but the improvement was somewhat greater when comparatively large amounts of bromate were added to them. Prefermentation of the germ caused its injurious effect on dough to disappear gradually as the time of prefermentation increased. The maximal improvement was reached after 4.5 hours' prefermentation and did not change with more extended prefermentation time. The presence of bromate in the fermenting germ suspension added to the improvement caused by prefermentation. Long prefermentation and/or long dough fermentation, particularly with bromate added at the dough stage, resulted in overmature loaves. The amino nitrogen and reducing matter content of aqueous germ suspensions increased with time of standing, and bromate tended to counteract these increases. The amino nitrogen of fermenting germ suspensions was found to be utilized by yeast. The reducing matter content of the suspensions, particularly when bromate was present, increased much less than it did in nonfermenting suspensions. While the nitroprusside reaction of prefermented germ suspensions became negative, their reducing matter content reached a level equal to or higher than that obtaining in the initial stage when the nitroprusside reaction was strongly positive. From the observation that bromate is much more effective at the dough stage than when added to a fermenting germ suspension, Smith and Geddes conclude that it exerts a direct action on flour proteins.

Laitinen and Sullivan (1941) applied the polarographic method to the study of oxidation-reduction systems in flour. They extracted

flours which had strongly responded to bromate with $0.1N$ potassium chloride or with acetate buffer solution. These extracts failed to give an anodic wave. The same negative result was obtained when the flour was treated with varying amounts of papain previous to extraction with an acetate buffer. An acetate buffer extract of wheat germ gave an anodic wave which was identified as that of reduced glutathione. Oxidation of glutathione in wheat germ resulted in a considerable decrease in the height of this wave. Unbuffered potassium chloride extracts of germ and bran yielded waves which disappeared on acidification, owing to a shift of the potential to more positive values. Extracts made from doughs (containing yeast, salt, sugar, flour, and water), with and without added potassium iodate, showed no distinctive waves. Yeast alone, extracted with acetate buffer, gave a small anodic wave. Flour extracts obtained by treating various flours with $N/1$KOH and then neutralizing with excess acetic acid so as to make an equimolecular acetate buffer, gave an anodic wave with a half-wave potential of -0.15 volt (S.C.E.). The anodic waves reached their maximum height after 24 hours' extraction time with KOH. These anodic waves could be made to disappear by addition of enough potassium iodate to make the solution $2 \times 10^{-4}M$. This shows that the anodic wave is due to a reducing substance. Further experiments with the various flour constituents showed that this reducing substance is released by the gluten. The authors further discuss the cathodic waves caused by the presence of potassium iodate in flour extract and the polarographic performances of various acids and amino acids, and they suggest that the formation of a mercury compound may be involved in the mechanism of the anodic wave. A buffered extract of alkali-treated gluten gave a positive mercury reaction on electrolysis.

Baker, Parker, and Mize (1942) studied the action of oxidants in bread doughs by measuring the flow of doughs and by investigating the different fractions (gluten, starch, "amylodextrin," washings) obtained in the gluten washing process. The authors reached the conclusion that either the gluten or the water soluble part of the dough is responsible for the effects on dough of oxidizing agents. They found the response of gluten to physical treatment to be the same as that of dough. The property of flow could be largely removed from gluten by washing, which seems to indicate that this property is associated with water soluble constituents. The effect of papain on gluten could be reversed by extensive washing. Water solubles obtained by repeated dispersal of gluten in the Waring Blendor with a 0.75% sodium chloride solution showed many of the properties of a proteose. A concentrated gluten wash solution had a softening effect on ordinary or rewashed

gluten but "tightened up" or left unchanged redispersed gluten. This was taken to show that constituents of the fraction removed by dispersal are responsible for the softening effect. It was this fraction also which was found to respond to the addition of sodium chlorite.

In view of the practical importance of the problems involved, and also for their general biochemical interest, the author thought it desirable to follow up, by quantitative methods, Hullett's and her own original experiments. The object of the present investigation was to determine (*a*) the effects of the germ proteases on the nitrogen distribution in the dough and (*b*) the effects of the —SH groups present in the germ on the —S—S— linkages of gluten.

The general plan of the study was to prepare doughs with and without addition of extracts from untreated and pretreated wheat germ, and after allowing these doughs to ferment or to stand unfermented, to wash the gluten from them and determine (*a*) the distribution of nitrogen between the soluble and insoluble fractions and (*b*) the cystine content of the washed gluten. It was anticipated that any changes effected by the action of germ proteases or germ —SH groups would be indicated by the values obtained in these determinations.

In the course of the experiments the idea emerged that dehydrogenases may play an important role in dough fermentation. In order to test this hypothesis the oxidation-reduction potential of germ extract was measured and a number of other tests were carried out.

Materials and Methods

Preparation of Doughs: Doughs were made from 25 g of a commercial New Zealand flour (straight run, 72% extraction) and 15 ml of dough liquid. No salt was added, because it was feared that it might interfere with the colloidal state of the proteins present in the dough extracts. When the doughs were to be fermented, 0.5 g of yeast was suspended in the dough liquid.

Dough Liquid: Each experiment consisted of a series of five doughs made with different dough liquids as follows:

(1) Extract from untreated wheat germ. This extract contained proteases and —SH groups, the determination of which will be discussed later.

(2) Heated and centrifuged extract from unfermented germ. This extract contained —SH groups but no enzymes.

(3) Extract from fermented germ. This extract contained enzymes but practically no —SH groups.

(4) Heated and centrifuged extract from fermented germ. This extract contained no enzymes and practically no —SH groups.

(5) Tap water.

The wheat germ used in these experiments was from a New Zealand flour mill and was used without any further comminution. Its content of pure germ, determined according to an unpublished method worked out by L. H. Bird at this Institute, was found to be approximately 64%.

Preparation of Wheat Germ Extracts Used as Dough Liquids: Extracts were prepared from this wheat germ in the following way: A mixture of 1 part of wheat germ and 2 parts of tap water, and another mixture of 1 part of wheat germ and 2 parts of yeast suspension containing 0.5 part of compressed yeast were allowed to stand for 4 hours at 30°C. They were then made up to their original weight, mixed with 4 parts of tap water, and centrifuged. Each centrifugate was divided into two portions, one of which was weighed, heated in a boiling waterbath for 3 minutes, cooled, made up to the original weight, and centrifuged. The dry solids contents of these 4 extracts were as follows:

(1) Extract from untreated germ.......................... 6.1%
(2) Heated and centrifuged extract from untreated germ...... 4.7%
(3) Extract from fermented germ.......................... 3.1%
(4) Heated and centrifuged extract from fermented germ...... 2.6%

Fifteen ml of each of these extracts was used as dough liquids without regard to the differences in their content of dry solids.

Washing of Gluten: After the doughs containing these extracts, or tap water, had stood for 3 hours at 27.8°C the gluten was washed from them by hand, 10 successive portions of 50 ml tap water being used for each sample. It was assumed that the total volume of combined washings would differ very little from 514 ml. This volume would be the difference between the total water, comprising (*a*) wash water, (*b*) flour moisture, (*c*) dough liquid, and the water remaining in the wet gluten.

When gluten was washed from unsalted doughs containing extracts from unfermented wheat germ, severe disintegration occurred and caused the bolting silk ordinarily used in the washing process to become blocked almost immediately after washing had begun. For this reason the bolting cloth was discarded altogether in all of the tests. Instead, each successive portion of washings was carefully decanted to minimize losses. The elimination of the bolting silk did, however, cause losses. While the duplicate determinations of mechanical loss agreed fairly well where water or extracts from fermented germ were the dough liquids, they showed much poorer agreement when the germ had not been fermented.

It was to be expected that proteolytic effects taking place in the dough would result in an increase of soluble nitrogen or of nonprotein

nitrogen in the washings, or in both of these factors. To detect such changes, nitrogen determinations in the washings before and after deproteinization would have been sufficient. Since, however, it was found that the use of extracts from unfermented wheat germ causes severe gluten disintegration during the washing process, it became advisable to use a method which would, in addition, estimate these losses. This was done by determining nitrogen (1) in the flour, (2) in the dough liquids, (3) in the filtered washings, and (4) in the unfiltered gluten hydrolysates. In order to include the humin nitrogen in the nitrogen determination, the gluten hydrolysates were not filtered. They were, however, well shaken before an aliquot was removed. The difference: $[(1) + (2)] - [(3) + (4)]$ gave mechanical losses. An attempt was made to determine nonprotein nitrogen in the washings by the method of Ayre and Anderson (1939) but owing to poor duplication it was abandoned.

Determination of —SH Groups as Reducing Matter in Germ Extracts: Reducing matter was determined by titration with standard potassium iodate solution at a pH of 2–3. When germ extracts are acidified to such a low pH most of their protein is precipitated. After the precipitate has been filtered and washed with acid, both the filtrate and the filter residue give a strong nitroprusside reaction. The —SH groups responsible for the positive reaction of the washed filter residue seem to be of the kind which Hopkins (1925) called "fixed" —SH groups, that is, —SH groups fixed to denatured protein. In this paper they will be referred to as protein —SH groups as distinct from the "soluble —SH groups" of the acid filtrate.[1]

Soluble —SH groups were determined by the following technique: To 10 ml of each of the wheat germ extracts 10 ml of 10% trichloracetic acid was added, the mixture was centrifuged, and the supernatant liquid decanted. The residue was washed and centrifuged twice with 10 ml of 3% trichloracetic acid, and the washings added to the original supernatant liquid. This solution, after addition of 2.5 ml of 5% potassium iodide solution and 1 ml of soluble starch, was titrated with $M/600$ potassium iodate solution.

To determine the protein —SH groups, the washed residue in the centrifuge flasks was dissolved in 10 ml of urea solution (1 : 1). This solution, acidified with a few drops of concentrated HCl, was titrated with KIO_3 in the same way as the solution containing the soluble —SH groups.

At first, the germ extracts were made completely ready for the doughs on the day before dough making and were kept in the refrigera-

[1] Hullett and Stern (1941) wrongly assumed that the positive nitroprusside reaction of the precipitated germ protein was due to adsorption.

tor overnight, but it soon became clear that the —SH content of the unheated extract decreased overnight, while that of the heated one remained unchanged. For this reason the unheated extracts which, for lack of time, had to be prepared the day before they were to be used, were kept in the refrigerator overnight but the heating and centrifuging were left until shortly before the particular doughs were made. The determination of —SH groups in each extract was made shortly after the corresponding dough had been mixed. Table III gives the results of these titrations.

In one series of germ extracts, reducing matter was determined by adding an excess of $0.005N$ iodine solution and back-titrating with standard thiosulfate solution. The values differed from those resulting from titration with iodate but they had the same trend.

Cystine Determination in Washed Gluten: Initially an attempt was made to determine —SH groups present in the filtered hydrolysates of the glutens washed from the doughs. As was to be expected, however, this approach was unsuccessful because, according to Lugg (1933), under the conditions of acid hydrolysis, the bulk of the cysteine is destroyed by formation of humin. Later, cystine was determined in these glutens because cystine should decrease with either reduction or oxidation of gluten —S—S— linkages. The technique of hydrolysis (with sulfuric acid) and colorimetry was that described by Mirsky and Anson (1935). The determination of extraneous reducing substances was carried out in the same way, except that (after Lugg, 1933) 0.5 ml of a 2.7% mercuric chloride solution was added in preparing the test solution for colorimetric reading.

Measurement of Oxidation-reduction Potentials in Germ Extracts: The oxidation-reduction potential of wheat germ has been measured by Potel (1935), who found E_h to be -169 mv. He prepared, in an atmosphere of pure nitrogen, the suspension of 1 part of the test material with 1.5 parts of phosphate buffer of pH 6.2, added 20 ml of toluene, and protected the mixture from air by covering it with a layer of vaseline oil. The measurement was made by means of a platinum electrode at 30°C. A stable equilibrium was reached after approximately 2 hours.

The measurements reported in this paper were carried out by means of a Coleman platinum electrode as follows: Five ml of unheated germ extract was adjusted to pH 6.6 with phosphate buffer, made up to 25 ml and a few drops of toluene were added. A stream of pure nitrogen was bubbled (at a speed of roughly 5 bubbles per second) through the electrode vessel containing the extract. The temperature was 17°C. Readings became constant after about 20 hours.

Response of Gluten to Heated and Unheated Germ Extract: Two

samples of gluten, each washed from the equivalent of 25 g of flour, were thoroughly kneaded for 5 minutes with 15 ml of germ extracts 1 and 2 respectively. After 5 minutes the gluten samples were washed with five successive portions of tap water, formed into balls, and placed on a glass sheet resting on graph paper. In order to avoid drying out, each sample was covered with a tumbler. Two diameters, at right angles to each other, of the gluten balls were measured by means of the graph paper immediately after washing and at various intervals thereafter and the areas calculated from the mean radii.

Detection of Dehydrogenases in Germ by the Thunberg Technique: One-half g of germ was mixed with 2 ml water and 0.4 ml of a 0.005% methylene blue solution in a Thunberg tube which was then evacuated and placed in a thermostat at 30°C. A second Thunberg tube containing the same quantities of wheat germ and water was kept in a boiling waterbath for 15 minutes and cooled to room temperature. Then 0.4 ml 0.005% methylene blue was added and the tube was evacuated and placed in the thermostat alongside the first tube.

Results

The results of the nitrogen determinations given in Table I show that the use of the extract from untreated germ as a dough liquid in

TABLE I

EFFECT OF WHEAT GERM EXTRACTS ON N DISTRIBUTION IN VARIOUS DOUGH FRACTIONS

	Dough liquid									
	Untreated germ extract		Heated, centrifuged extract from unfermented germ		Extract from fermented germ		Heated, centrifuged extract from fermented germ		Water	
	Dough									
	No yeast	Yeast	No yeast	Yeast	No yeast	Yeast	No yeast	Yeast	No yeast	Yeast
N in 25 g flour, mg	346	346	346	346	346	346	346	346	346	346
N in 15 ml dough liquid, mg	60	60	24	24	32	32	22	22	—	—
Total N in dough, mg	406	406	370	370	378	378	368	368	346	346
Soluble N in washings, mg	93	106	81	102	83	104	77	95	58	93
N in gluten hydrolysate, mg	173	101	199	102	266	217	257	201	255	204
Lost N, mg	140	199	90	166	29	57	34	72	33	49
Lost total N[1] %	34.5	49.0	24.3	44.9	7.7	15.1	9.2	19.5	9.5	14.2
Lost gluten N[2] %	44.7	66.3	31.1	61.9	9.8	20.8	11.7	26.4	11.4	19.3

[1] Loss of gluten nitrogen expressed as percent of total nitrogen.
[2] Loss of gluten nitrogen expressed as percent of gluten nitrogen.

unsalted nonyeasted doughs led to a severe breakdown of the gluten; this is indicated by high mechanical loss in gluten washing. The loss was only slightly lessened when the extracts were heated and centrifuged. However, in those doughs for which water or extracts from fermented germ had been used the loss of gluten was very much smaller; this held whether or not extracts had been heated and centrifuged. These results, therefore, show that fermentation of germ greatly lessens the disruptive effect of its extract on the dough. In the yeasted doughs the losses were much higher throughout than in the nonyeasted series, but the trend was the same.

Table I shows further that the soluble nitrogen in the washings from the yeasted blank is higher by 60% than that in the washings from the nonyeasted blank. Amos (1931), Freilich and Frey (1943), and Shen and Geddes (1942) have shown that nitrogen compounds are utilized by actively fermenting yeast, and for this reason concluded that the soluble nitrogen contained in a fermenting dough is unsuitable as a measure of proteolytic activity. In the present experiments, notwithstanding the partial consumption of soluble nitrogen by the yeast, an increase of soluble nitrogen occurred. This observation confirmed similar ones made by other workers.

The inclusion of wheat germ extracts in the dough, whether or not the germ had been fermented and/or the extracts heated, led to higher figures for soluble nitrogen in the washings, as compared with the blank. That no real increase, but in fact a decrease, takes place can be seen when allowance is made for the soluble nitrogen present in the dough liquids (Table II).

TABLE II

Effect of Wheat Germ Extracts on the Formation of Soluble Nitrogen in Doughs

	Dough liquid									
	Untreated germ extract		Heated, centrifuged extract from unfermented germ		Extract from fermented germ		Heated, centrifuged extract from fermented germ		Water	
	Dough									
	No yeast	Yeast	No yeast	Yeast	No yeast	Yeast	No yeast	Yeast	No yeast	Yeast
Sum of soluble N in blank + dough liquid, mg	118	153	82	117	90	125	80	115	58	93
Soluble N found, mg	93	106	81	102	83	104	77	95	58	93

The values in Table II show that the incorporation of germ extract into dough did not result in making soluble any proteins that were insoluble in the blank. The striking fact that there was an actual decrease of soluble nitrogen when extracts of untreated germ were used, and that a similar tendency was noticeable in the case of the other germ extracts, will be discussed later. That the flour and the germ did, however, contain proteases is shown from the following evidence: Determination of protease activity as described in Cereal Laboratory Methods (4th ed. 1941) gave increases of 12 mg amino nitrogen per 100 g of flour, and 42 mg per 100 g of germ after a digestion of 24 hours at 40°C. The soluble nitrogen of a nonyeasted dough made with tap water increased by 15 mg during a standing period of 3 hours at 27.8°C.

The data in Table III show that when extracts from unfermented germ are heated and centrifuged the bulk of the protein carrying the

TABLE III

Soluble and Masked —SH Groups Present in Wheat Germ Extracts
(Expressed in ml $M/600$ KIO_3 consumed by 15 ml dough liquid)

	Nature of dough liquid[1]			
	Untreated germ extract	Heated, centrifuged extract from unfermented germ	Extract from fermented germ	Heated, centrifuged extract from fermented germ
Soluble —SH groups	2.01	1.86	0.12	0.17
Protein —SH groups	2.30	0.22	0.60	0.07
Total	4.31	2.08	0.72	0.24

[1] The headings containing the word "centrifuged" refer to the centrifuging carried out to remove protein coagulated by heating. They do not refer to the centrifuging which all extracts underwent after addition of trichloracetic acid.

protein —SH groups is removed, but the soluble —SH groups remain practically undiminished. It can further be seen from these figures that a 4 hours' fermentation of germ at 27.8°C results in the disappearance of the bulk of the soluble —SH groups and in a considerable decrease of the protein —SH groups in the extracts. When extracts from fermented germ are heated and centrifuged, the protein —SH groups are removed. Thus, fermentation, heating, and centrifuging results in a far-reaching decrease of both soluble and protein —SH groups.

The removal by heating and centrifuging of the bulk of the protein —SH groups from an extract of unfermented germ did not appreciably decrease its injurious effect on dough, although its total content of

reducing matter had dropped to approximately half of its original value. This indicates that only the soluble —SH groups cause dough deterioration. Table IV, combined from Tables I and III, shows the

TABLE IV

RELATION BETWEEN SOLUBLE —SH GROUPS IN DOUGH LIQUIDS AND LOSSES OF GLUTEN IN THE WASHING PROCESS

	Dough liquid									
	Untreated germ extract		Heated, centrifuged extract from unfermented germ		Extract from fermented germ		Heated, centrifuged extract from fermented germ		Water	
	Dough									
	No yeast	Yeast	No yeast	Yeast	No yeast	Yeast	No yeast	Yeast	No yeast	Yeast
Soluble —SH groups	2.01	2.01	1.86	1.86	0.12	0.12	0.17	0.17	—	—
Loss of gluten-N in washing, %	44.7	66.3	31.1	61.9	9.8	20.8	11.7	26.4	11.4	19.3

correlation between soluble —SH groups in the dough liquid and gluten disintegration in terms of loss in washing.

TABLE V

EFFECT OF WHEAT GERM EXTRACTS ON CYSTINE CONTENT OF GLUTEN

	Dough liquid									
	Untreated germ extract		Heated, centrifuged extract from unfermented germ		Extract from fermented germ		Heated, centrifuged extract from fermented germ		Water	
	Dough									
	No yeast	Yeast	No yeast	Yeast	No yeast	Yeast	No yeast	Yeast	No yeast	Yeast
Cystine in recovered gluten, mg	30.8	13.0	33.6	14.4	48.5	30.2	47.3	29.2	46.1	29.7
Cystine in total gluten, mg	55.7	38.6	48.8	37.8	53.8	38.1	53.6	39.7	52.0	36.8

The data in Table V show that fermentation, but not addition of germ extract, causes a significant decrease in the cystine content of gluten.

The values in the second line of Table V were computed on the assumption that the cystine content of the coherent part of the gluten

is the same as that of the disintegrated fraction. If this is true, the cystine determined in the hydrolysate of the coherent fraction should bear the same relation to the cystine, present but not determined, in the disintegrated part as the nitrogen contents of these two gluten fractions bear to each other. The total cystine content of gluten would thus be: $\frac{\text{cystine found}}{100 - \%\ \text{lost gluten}}$. Where the figures calculated on this basis differ significantly from those referring to the nonyeasted blank, either a change in the cystine content of the gluten or of cystine distribution (between coherent and disintegrated fractions) must have taken place as a consequence of the particular treatment.

The results of the experiments, which related to the effects of dehydrogenases, were as follows:

The oxidation-reduction potential of untreated wheat germ extract was found to be -115 to -130 mv; that of fermented wheat germ extract -150 to -160 mv.

Wheat germ, tested by the Thunberg technique, was found to contain dehydrogenases; the unheated germ suspension decolorized methylene blue in 5 minutes at 30°C whereas a similar suspension which had been previously heated in a boiling waterbath for 15 minutes did not decolorize methylene blue.

When gluten was treated with the germ extracts 1 and 2, the rates of flow as reflected by the relative areas of the gluten surfaces in contact with graph paper were as follows:

Areas	Immediately after washing	After 1 hr	After 2 hr	After 3 hr	After 5 hr	After 6 hr
Extract 1 (untreated)	64	82	95	113	118	133
Extract 2 (heated, centrifuged)	78	118	148	165	177	177

Discussion

The doughs used in the experimental part of this investigation are not comparable to ordinary bread doughs, mainly because they do not contain salt. Therefore the following views, which were suggested by the experimental results, will have to be checked by evidence obtained with ordinary bread doughs.

The determination of the nitrogen distribution in doughs revealed the interesting fact that neither the addition of proteases nor of —SH groups resulted in an increase of soluble nitrogen but that, on the

contrary, there was a decrease of soluble nitrogen. This latter fact can only be explained by the assumption that the gluten incorporated some of the protein from the dough liquids. Something of the same nature seems to take place in the well-known case where an increased yield of gluten is obtained by washing gluten from a mixture of wheat and rye flour.

Shen and Geddes (1942) give a survey of the evidence used by various workers in support of Jørgensen's hypothesis (activation of flour proteases by —SH groups) and of the hypothesis that —SH groups exert a direct action on gluten. It may be of interest to investigate how the results reported in this paper and some of the earlier evidence fit in with either of these hypotheses.

If Jørgensen's view is correct, the injurious effect of unheated germ extract could be explained as a consequence of adding —SH groups and germ proteases to the dough. The injurious effect of heated germ extract may also be attributed to —SH groups acting on the flour proteases, but one would expect that the inactivation of the germ protease in the dough liquid would result in lessening the damage to some extent. Actually, the damage (expressed in terms of loss in washing and increase of soluble nitrogen) caused by the heated germ extract was less severe than that due to unheated germ extract. Also, the good characteristics of dough made with germ extracts (heated or unheated) from fermented germ may be explained by the absence of —SH groups. This experiment, however, cannot be regarded as convincing evidence in favor of Jørgensen's hypothesis, for the reason that it supports the direct-action hypothesis equally well.

This latter hypothesis does not deny that proteases are present in flour and that they belong to the papain type which is activated by —SH groups but it claims that —SH groups also have a direct effect on gluten; *e.g.* when gluten is treated with cysteine. On the basis of this hypothesis, the effects on doughs of the wheat germ extracts described in the experimental part may be explained as follows: Unheated extract from untreated germ has a proteolytic plus a direct effect; after heating it has a direct effect only. In extracts from fermented germ the protease is inactivated as no —SH groups are present; similarly after heating, the protease is destroyed and no —SH groups are present. Thus, this experiment, while giving information on changes in gluten structure, is not helpful in judging the validity of either of the two hypotheses.

One of the main objections to Jørgensen's hypothesis is the fact that prolonged fermentation of doughs injured by —SH compounds leads to a very considerable improvement of dough and loaf characteristics. Such an improvement with time is not compatible with the

idea that the initial damage is caused by proteases. As Hullett (1940) was the first to suggest, the elimination of —SH groups from a fermenting dough may be an important part of the ordinary dough-ripening process. The work of Smith and Geddes (1942) also makes it appear probable that the improving effect of long fermentation is due to the destruction of —SH groups. While this gradual disappearance of —SH groups may cause a gradual inactivation of proteases, it is quite unlikely to reverse chemical changes brought about by proteolysis in the earlier stages of fermentation.

If, on the other hand, one tries to interpret the observations on the basis of the direct-action hypothesis, one arrives at the conclusion that this direct action must be of such a nature that, by the oxidation of the —SH groups, it allows gluten structure to be restored to a normal condition. Otherwise the improvement of dough characteristics with length of fermentation could hardly be explained.

The abnormal response of gluten to treatment with unheated and heated germ extracts shows that a heat-labile factor has a tightening effect on gluten, whether damaged by —SH groups or not. Obviously, heating the germ extract destroys some factor which favorably affects gluten structure or lessens the damage due to —SH groups. Sullivan, Near, and Foley (1936) found that wheat germ stored in closed containers at relatively high moisture content loses its injurious effect. Hullett and Stern (1941) and Smith and Geddes (1942) showed that fermentation causes a much more rapid elimination of these damaging properties, and the first-named authors found that an enzyme contained in the germ was essential in this process. Both storage and fermentation of wheat germ are accompanied by the disappearance of the nitroprusside reaction, and in both cases it was found impossible to restore the nitroprusside reaction by means of reducing agents.

On the basis of these findings the author assumed that the enzyme responsible for the elimination from germ of the —SH groups is a dehydrogenase and that the oxidation of the —SH groups proceeds beyond the —S—S— stage. The observation that cyanide does not inhibit the destruction of —SH groups by fermentation (Hullett and Stern, 1941) fits in with the assumption that a dehydrogenase is effective in this process.

Dehydrogenases are present in many seeds, as Thunberg has found. The present study indicates that they are present in wheat germ in considerable amount. Since every flour contains some germ, the amount varying with the length of extraction, it seems reasonable to expect that mechanisms analogous to those playing a part during storage and fermentation may be active in flour and dough. Fer-

mentation speeds up the otherwise slow action of the dehydrogenases. Nothing is known so far about the mechanism of this acceleration except that the glutathione present in the yeast cell enters into it, as was found by Hullett and Stern (1941).

By oxidizing —SH compounds, the dehydrogenases would bring about the aging of green flour and the maturing of dough. The gradual elimination of —SH groups by dehydrogenases would explain the improving effect of prolonged fermentation of low-grade flours. In the gluten experiment the presence of dehydrogenase in the unheated extract would tend partly to offset the damage caused by —SH groups and proteases.[2]

Sullivan *et al* (1940) suggested that dough fermentation, reducing and oxidizing agents, and physical manipulation may bring about changes in the sulfur linkages of the gluten proteins. The results reported above showed that, under the conditions applied, fermentation produced a significant decrease of cystine in the gluten, whereas the presence of wheat germ extracts rich in —SH groups failed to produce a significant change in the cystine content of the gluten.

As has been discussed above, it is difficult to explain the improving effect of prolonged fermentation on dough made from low-grade flours unless one assumes the direct action of —SH groups to be of such a nature that it allows gluten structure to be restored to normal condition. The cystine determinations reported in this paper indicate that the addition of —SH compounds had no significant effect on the —S—S— linkages of the gluten. This shows that the —S—S— linkages were not involved in whatever reaction may have taken place owing to the direct action of —SH groups. The fact that dough fermentation leads to a considerable decrease of cystine in gluten confirms the views of those investigators who maintain that yeast is not only a leavening agent but produces fundamental biochemical changes in the dough.

Summary

Wheat germ extracts contain soluble —SH groups and —SH groups attached to protein.

The distribution of soluble and protein —SH groups in heated and unheated extracts from unfermented and fermented wheat germ is reported.

The soluble —SH groups were found to cause the injurious effect of wheat germ extract on dough.

Fermentation of wheat germ decreases its content of soluble and protein —SH groups.

[2] This experiment also suggests that the dehydrogenase must have exerted a considerable effect within the 5-minute contact of the gluten with the germ extracts.

Extracts from unfermented wheat germ, when added to unsalted dough, cause severe gluten disintegration on washing.

Dough fermentation increases the gluten disintegration in the blank doughs as well as in those containing germ extracts.

There is no increase, but an actual decrease, of soluble nitrogen in doughs containing extracts from unfermented germ.

Dough fermentation leads to a decrease of cystine in the gluten, but —SH groups did not significantly affect the cystine content of the gluten.

Aging of flour and maturing of dough is assumed to be connected with the oxidation of the —SH groups of germ particles contained in the flour.

The oxidation of —SH groups in stored flour and fermenting dough is attributed to dehydrogenases, and the presence of dehydrogenases in wheat germ is demonstrated.

Reasons are given for the assumption that the injurious effect of —SH groups is direct but of such a nature that it will allow the gluten to be restored to normal, that is, to the condition obtaining in fermented loaves made from high grade flours.

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THE ACTION OF OXIDIZING AGENTS ON SULFHYDRYL COMPOUNDS IN DOUGH

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This paper reports further studies on the effects of oxidizing agents on refined flour and dough. The work has been guided by our previous researches (Baker, Parker, and Mize, 1942; Baker, Mize, and Parker, 1943), in which it was found that oxidizing agents cause changes in puncture strength of gluten and in properties of soluble pentosan and soluble protein. While, admittedly, effects of oxidizing agents upon starch, other carbohydrates, and lipoids should be studied further, it seemed that continued studies of the proteins were more promising, especially since it had been found that glutathione lowered the resistance of gluten to puncture and influenced the viscosity of water soluble pentosans. Also, as many workers have shown (Balls and Hale, 1936; Jørgensen, 1936; Sullivan, Howe, and Schmalz, 1936, 1937; Ford and Maiden, 1938; Freilich and Frey, 1939; Ziegler, 1940, 1940a; Hullett and Stern, 1941; and others), glutathione profoundly affects dough and its bread making properties.

Sullivan, Howe, and Schmalz (1936) isolated glutathione from wheat germ but reported that patent flours contained no glutathione as indicated by the nitroprusside test. Hence, it could be assumed that there were no other available sulfhydryl compounds present in patent flour. Balls and Hale (1936) have shown that a positive nitroprusside

test can be obtained from petroleum ether extracts of patent flour and have suggested that sulfhydryl groups may be present in flour protein. They have also isolated a water soluble sulfur-containing polypeptide from the petroleum ether extract. These observations, together with the fact that sulfhydryl compounds are easily oxidized, suggest that the baking properties of flours may be influenced by their sulfhydryl content, particularly in regard to their behavior toward oxidizing agents.

Greenstein (1938) and Anson (1941) have published methods for the determination of -SH groups in proteins. Greenstein (1938) showed that sulfhydryl groups, which cannot be detected in certain native proteins (such as egg albumin, edestin, excelsin, and globins), are present in these proteins after denaturation with guanidine hydrochloride and urea, or their derivatives. He titrated the -SH groups with porphyrindin as the oxidizing agent employing sodium nitroprusside as the end point indicator. Anson used either sodium tetrathionate or potassium ferricyanide in place of porphyrindin. The end point is the least amount of ferricyanide or tetrathionate which will prevent the formation of the pink color, thus indicating the amount required to oxidize the sulfhydryl groups.

In the present study, the sulfhydryl contents of aqueous extracts and glutens, prepared from highly refined flours of varying strength, were determined. The effect of oxidizing agents on the -SH content of these flour and dough fractions was investigated. The puncture strength of the glutens was also determined to ascertain whether any relation exists between gluten strength and -SH content.

Experimental

Determination of sulfhydryl groups. Preliminary studies showed that the use of guanidine hydrochloride as a solvent in determining the sulfhydryl content of flours and doughs was unsatisfactory. The starch present gave a highly viscous solution which made mixing of the reagents difficult and also interfered with the color reaction so that the end point could not be determined. A modified Anson (1941) method, however, was found to be satisfactory when applied to concentrated extracts of flour or dough (flour-water ratio, 1 : 2) prepared as described by Baker, Mize, and Parker (1943). It also gave very good results with aqueous dispersions of gluten. To disperse the glutens for sulfhydryl determinations it was necessary to use less salt than formerly described; the doughs were mixed with 0.75% NaCl solution and the glutens washed therefrom with 0.15% NaCl solution. The glutens thus prepared from refined flours could be dispersed in distilled water with the Waring Blendor to a stable milklike emulsion of 2.0% protein content, which made accurate sampling possible.

The method of determining -SH groups, as used in this study, is outlined below.

Reagents: 1. Buffer—equal parts $1.0M$ disodium phosphate, and $1.0M$ monosodium phosphate.
2. Potassium cyanide solution, $0.1N$.
3. Guanidine hydrochloride—highest purity.
4. Sodium nitroprusside solution, 5%. This is best prepared from ground sodium nitroprusside. It should be freshly made each day and stored at 0° C away from direct sunlight.
5. Concentrated ammonium hydroxide, 27%.
6. Potassium ferricyanide; standard solution series ($0.000025M$ to $0.0008M$, in increments of $0.0001M$).

Procedure: The titration of the -SH groups is carried out by means of a series of test tubes in each of which is placed 0.5 ml of flour extract or gluten suspension, 0.1 ml of buffer solution, 0.05 ml of $0.1N$ potassium cyanide, and 0.5 ml of standard potassium ferricyanide solutions of varying strengths. Next, 1.2 g of guanidine hydrochloride (Eastman) is added; the tubes are shaken lightly to dissolve the guanidine hydrochloride, and then immersed in a water bath at 37° C for 3 minutes, followed by cooling in an ice bath. When cool, 0.05 ml of nitroprusside reagent is added, shaken, and followed by 0.05 ml of 27% NH_4OH. By selection of the proper pipette tip 0.05 ml can be delivered with one drop. The contents of the tubes are now mixed and the end point is taken as the concentration of ferricyanide where the pink color just fails to develop. From the quantity of ferricyanide required, as checked against glutathione standards, the -SH, or glutathione, content of the flour extract can be calculated.

Since the -SH values are in the lower range of Anson's scale, great accuracy cannot be expected. Although the method is fairly accurate and reproducible when applied to soluble extracts or dispersed glutens from refined flours, lower grade flour extracts or gluten suspensions appear to have materials present which interfere with the end point, causing rapid fading and off-shades of color. A further limitation of the method was found in studying fermented doughs and glutens prepared therefrom; yeast or its by-products interferes somewhat with the duplication of results. Anson prescribes very dilute cyanide to remove interfering effects of heavy metals. We have used this precaution in our determinations. The small amount of cyanide used does not increase the readings, indicating that there is no splitting of S-S groups. Anson recommends the use of reagents of highest purity for sharpest end points.

Action of oxidizing agents in flour. The sulfhydryl contents of water extracts, hand-washed glutens, and purified glutens from a series of commercial flours of widely varying strength are given in Table I, together with the puncture strength of the glutens. The nitrogen contents of the fractions were determined and the -SH values are expressed on a unit protein basis.[1]

The -SH content per unit of protein in the water extract of the different flours is variable. The glutathione content of the solubles

[1] This method of expressing our results is not intended to imply that the -SH so recorded is thus part of the protein compound.

TABLE I

SULFHYDRYL CONTENT OF WATER EXTRACTS AND GLUTENS FROM VARIOUS WHEAT FLOURS

Description of flour	Ash	Protein	Soluble protein	-SH per gram protein			Grams to puncture gluten	
				Water extract	Hand-washed gluten	Purified gluten	Gluten washed in 0.15% NaCl	Gluten purified in 0.15% NaCl
	%	%	%	mg	mg	mg	g	g
Montana	0.51	14.2	1.9	1.22	0.22	0.13	10.0	19.7
Nebraska	0.40	11.9	1.7	0.88	0.20	0.09	8.5	16.7
Durum	0.61	12.0	1.7	1.14	0.19	0.12	4.8	10.1
No. Spring	0.39	12.9	1.6	0.83	0.17	0.10	10.5	24.9
Soft Winter	0.31	8.2	1.3	0.73	0.12	0.10	10.7	19.5
Kansas	0.39	12.2	1.7	0.89	0.17	0.10	6.6	14.2
Pacific Pat.	0.41	10.5	1.5	0.87	0.15	0.12	6.1	14.6
Pacific Clear	0.73	12.2	2.5	1.31	—	—	4.9	11.6
Idaho	0.41	11.2	2.0	1.01	0.12	0.05	7.2	14.5

doubtless depends upon the amount of germ milled into the flour. There may be other -SH bearing compounds present, such as polypeptides, which are intermediate between glutathione and protein. The relative amounts of such compounds could account for the varying sulfhydryl content per unit protein of these soluble extracts.

The hand-washed glutens show a considerable variation in sulfhydryl content. In purified gluten, since dispersion washing by the method previously described by us has further removed soluble sulfhydryl compounds there appears to be -SH which cannot be removed by washing. There is no relation between the glutens and their sulfhydryl contents or the sulfhydryl content of the water extract.

Table II shows the effect of relatively large treatments of the Montana flour listed in Table I with nitrogen trichloride and chlorine on the sulfhydryl content and puncture strength of the glutens. These oxidizing agents lowered the sulfhydryl of the soluble extract about 20%, and of the purified gluten over 30%. If one assumes that glutathione is the compound in the soluble extract which has been oxidized, the decrease in -SH is equivalent to 36 ppm of glutathione. More than this amount is actually oxidized in the flour because none of the soluble -SH which is retained by the starch or gluten is accounted for by this calculation. The difference in -SH content of the hand-washed glutens from untreated and heavily nitrogen-trichloride-treated flour is equivalent to 56 ppm of glutathione. This value, together with the calculated equivalent glutathione in the water extract, totals about 92 ppm glutathione and is sufficient to account for most of the changes observed in flour treated by flour oxidants.

TABLE II

EFFECT OF FLOUR OXIDATION ON SULFHYDRYL CONTENT OF WATER EXTRACT AND GLUTEN

Treatment	-SH per gram protein			Grams to puncture gluten	
	Water extract	Hand-washed gluten	Purified gluten	Gluten washed in 0.15% NaCl	Gluten purified in 0.15% NaCl
	mg	*mg*	*mg*	*g*	*g*
MONTANA FLOUR					
Untreated	1.22	0.22	0.13	10.0	19.7
Nitrogen trichloride, 6 g/bbl	0.95	0.16	0.09	9.8	20.0
Chlorine, 2 oz/bbl	0.98	0.17	0.09	9.0	21.9
IDAHO FLOUR—NATURAL AGE					
Stored 6 mo refrigerator	1.01	—	0.05	7.2	14.5
Stored 6 mo room temp	0.84	0.12	0.04	6.5	14.9

Experimentally, we have found that 50 ppm of glutathione will cause a dough from an optimum nitrogen-trichloride-treated flour to revert to the "green" characteristics of its untreated control, while 100 ppm of glutathione will usually produce a "green" dough when added to an over-treated flour.

The hand-washed gluten from an untreated flour, upon purification by dispersing four times in a Waring Blendor, has its reactive -SH lowered from 0.22 to 0.13 mg/g of gluten; in treated flours it has been lowered to 0.09 mg. The residual -SH is not free glutathione since glutathione added to gluten or to flour can be removed by thorough dispersion washing. The reaction of flour oxidants upon the sulfhydryl of purified glutens might account for the alteration of the gluten properties. However, the puncture values of such glutens, as shown in the last column, are not altered appreciably by the treatment.

In Table II the naturally aged flours show changes in -SH similar to those produced by nitrogen trichloride and chlorine.

There are doubtless other effects of oxidizing gases on the flour than those indicated by these -SH analyses. The petroleum-ether-soluble -SH-containing compound reported by Balls and Hale (1940) was found by them in sufficient amount to account for the above changes in -SH in flour bleaching.

Extraction of flour with volatile fat solvents lowered the sulfhydryl content of the flour fractions to approximately the same extent as is

shown by the gas-treated flours in Table II. However, treatment of such extracted flours with dough oxidants shows a further lowering of the -SH content of both water extract and gluten, indicating that there are reactive sulfur compounds in flour other than those which are removed by fat solvents.

Action of oxygen on doughs. A study of the action of molecular oxygen is shown in Table III. The Montana flour referred to in

TABLE III

EFFECT OF MIXING DOUGHS IN OXYGEN AND CARBON DIOXIDE ON SULFHYDRYL CONTENT OF WATER EXTRACT AND GLUTEN
(Montana Wheat Flour)

Duration of mix	Gas	-SH per gram protein			Grams to puncture gluten	
		Water extract	Hand-washed gluten	Purified gluten	Gluten washed in 0.15% NaCl	Gluten purified in 0.15% NaCl
min		*mg*	*mg*	*mg*	*g*	*g*
8	CO_2	1.22	0.22	0.13	10.0	19.7
8	O_2	1.07	0.21	0.09	11.9	23.1
16	O_2	0.91	0.14	0.09	12.4	22.5
24	O_2	0.84	0.13	0.09	12.4	23.3
40	O_2	0.84	0.15	0.09	10.0	17.5
40	CO_2	1.12	0.22	0.13	10.8	20.5

Table II was mixed in the presence of pure oxygen for periods of time varying from 8 to 40 minutes in a covered McDuffy mixing bowl. Similar mixings in carbon dioxide for an 8- and a 40-minute period indicate that the mixing itself had substantially no effect upon the sulfhydryl of the water extract or of the glutens. Oxygens, however, progressively lowered the -SH content of the water extract as the mixing was continued up to 24 minutes. Further mixing produced no change, indicating that the -SH in the water-soluble portion of the dough is susceptible to attack to a limited degree. The first 8 minutes of mixing in oxygen decreased the -SH content of the gluten, after which no further decrease occurred.

The puncture values reached a maximum after 8 minutes of mixing in oxygen, and remained substantially constant up to and through 24 minutes of mixing, *but* mixing for 40 minutes materially lowered the strength of the gluten. The sulfhydryl figures, however, do not suggest this change. Doubtless, the lowering of the protein strength is due to some action other than on the -SH groups. These data again indicate that there is an oxidizable form of sulfur in gluten which is not readily removed by washing.

Action of sodium chlorite in doughs. A further study of the effect of dough oxidants is shown in Table IV. Dough was mixed for 8 minutes

TABLE IV

EFFECT OF DOUGH OXIDATION WITH SODIUM CHLORITE ON SULFHYDRYL CONTENT OF WATER EXTRACT AND GLUTEN
(Doughs Prepared from Kansas Flour)

Sodium chlorite	-SH per gram protein			Grams to puncture gluten	
	Water extract	Hand-washed gluten	Purified gluten	Gluten washed in 0.15% NaCl	Gluten purified in 0.15% NaCl
ppm	*mg*	*mg*	*mg*	*g*	*g*
0	0.89	0.17	0.10	6.6	14.2
2.5	0.84	0.13	0.09	5.7	17.5
5.0	0.79	0.14	0.09	7.4	18.2
10.0	0.79	0.16	0.09	8.2	15.7
20.0	0.79	0.14	0.09	6.1	14.1
40.0	0.73	0.17	0.09	2.8	9.9
80.0	0.61	0.18	0.09	1.8	7.0

in the presence of sodium chlorite in amounts up to 80 ppm. Our supply of the Montana flour being exhausted, these experiments were performed on a Kansas flour. This flour contained less water soluble -SH than the Montana flour and also less -SH in the hand-washed and purified gluten. Sodium chlorite, the oxidant used in this experiment, decreased sulfhydryl content of the water extract less than did the oxygen or the gas treatments with the Montana flour used in the previous two experiments. This Kansas flour apparently has a lower proportion of oxidizable sulfur in the water extract than the Montana flour. A powerful oxidizing agent, such as sodium chlorite, would be expected to produce a greater effect than molecular oxygen or flour oxidants.

As in the previous experiment, an -SH level in the water extract is reached which is decreased by further oxidation with difficulty. Over the range of treatments from 5 ppm to 20 ppm no change occurred and the puncture strength of the glutens maintained a fairly high figure. Heavier treatments with sodium chlorite slightly lowered the -SH content of the water extract, and at the same time lowered the puncture strength of the gluten. It is difficult to associate puncture strength of gluten with the constituents of the water extract unless the strength of the former is determined by adsorbed materials. However, the thoroughly washed, purified glutens from this flour suggest no such change in the adsorbed materials. Neither is there much effect of the chlorite upon the sulfhydryl of the glutens. With this flour, nearly all the sulfhydryl sulfur could be washed from the gluten, resulting in constant values for the purified gluten throughout the whole range of the treatments. This value is only slightly lower than the -SH in the untreated purified gluten. The purified glutens of these two flours

(Montana and Kansas) indicate a difference in the amount of -SH adsorbed in the gluten and in the ease with which the -SH may be removed by washing. After oxidation and thorough washing, the amount of residual -SH in the glutens from the two flours reaches almost the same value over the total range of the respective treatments, indicating that the amount of nonoxidizable -SH in the purified glutens in these widely different flours is approximately the same. Other flours may have glutens which retain less -SH as shown by the naturally aged Idaho flour in Table II.

The baking properties of these doughs appear to be associated more definitely with the strength of the gluten than with their sulfhydryl content. Gluten puncture strength was increased by the use of sodium chlorite up to 5 ppm and then steadily decreased with increasing dosages. These changes in protein strength closely parallel the changes in the baking properties of straight doughs given this range of treatment. Other methods of baking, such as sponge doughs and no-dough-time doughs, need the higher levels of sodium chlorite where some weakening of the gluten has occurred. Further research is needed to determine the chemical reactions responsible for the changes in the physical properties of the gluten which result from treatment with oxidizing agents.

As previously mentioned, difficulty was encountered in obtaining satisfactory results on fermented doughs. Disintegrated yeast which does not separate from the supernatants upon centrifuging, and yeast which remains in the gluten throughout the washing process, apparently causes this difficulty. In spite of these difficulties, it has been found that during fermentation bromate alters the sulfhydryl content of the water extract and the puncture strength of the gluten in a manner similar to the medium chlorite treatments shown in Table IV. Fermentation alone, as used in bread making, lowers the sulfhydryl content of the water extract to a small degree. Shen and Geddes (1942) found that the total reducing matter content of extracts prepared from nonfermenting doughs increased more with time than that of fermenting doughs; bromate had a marked depressing effect which was more pronounced the longer the fermentation.

Hullett and Stern (1941) report that extended fermentation will completely remove glutathione from germ. The slight decrease in -SH content of water extracts prepared from fermented doughs made from refined flours indicates that there is little glutathione in these flours. Hence, the change in -SH by oxidation must be due to the action of the oxidizing agents upon -SH bearing material other than glutathione. This is in agreement with the work of Sullivan, Howe, and Schmalz (1936), in which they were unable to separate glutathione from flour.

Discussion

The nitroprusside test for indicating sulfhydryl groups is not sufficiently sensitive to indicate clearly their presence in the native flour fractions, as separated by our procedures, *viz.*, the water solubles, the crude gluten, and the purified gluten. Active sulfhydryl groups must be present in these fractions since flour and dough oxidants decrease the amounts of sulfhydryl found when determined after denaturing with guanidine hydrochloride. We have found these commercially used oxidizing compounds capable of lowering the amount of -SH found when determined in the guanidine hydrochloride solution of the three fractions of all flours. These reactions indicate that the -SH is reactive in the native flour when contacted by certain flour bleaching agents or dough improvers or by molecular oxygen acting in either flour or dough. This activity of -SH groups has also been shown by Anson (1941). He found upon treating solutions of native proteins with iodine in potassium iodide that complete oxidation of the active -SH groups was obtained, as determined after denaturing.

The purified glutens from all but two of the flours studied contain approximately the same amount of -SH. The method of determining -SH is not sufficiently accurate at these low levels nor have enough flours been studied to draw any conclusions from this apparent uniformity of composition. Nevertheless, the two exceptions shown above appear sufficient to void any conclusion, for if purified glutens were constant in their sulfhydryl composition there would be no exceptions.

It appears most likely that the active -SH groups in purified gluten are in adsorbed compounds and so strongly held that the extensive washing processes which we have used do not entirely remove them. Differences in the amount of such adsorbed compounds would lead to the variation found in the composition of the purified gluten.

Summary

Sulfhydryl content of concentrated aqueous extracts and gluten dispersions prepared from highly refined flours may be determined by a modified Anson method. With refined flours of widely varying strength, the -SH values per gram protein ranged from 0.73 to 1.22 mg for aqueous extracts; from 0.12 to 0.22 mg for hand-washed glutens; and from 0.05 to 0.13 mg for purified glutens. No consistent relationship was found between the -SH content of the three flour fractions of various flours or between their -SH content and gluten strength. A large fraction of the -SH in the glutens could not be removed by repeated washings. Since added glutathione may be readily washed out, a considerable proportion of the sulfhydryl groups of flour is

apparently present in more complex cysteine combinations. These sulfhydryl compounds do not appear to be an integral part of the molecular structure of the gluten proteins, since the -SH content of the purified glutens varied widely. The sulfhydryl bearing compounds probably are of many compositions and include glutathione, the fat soluble sulfur bearing compound of Balls and Hale, and doubtless other compounds of more complex structure which are more strongly adsorbed by the flour proteins.

Treatment of flour with chlorine and nitrogen trichloride, mixing doughs in oxygen or with sodium chlorite, fermenting doughs with or without bromate, and natural aging of flour lowered the -SH content of water extracts and glutens prepared therefrom. The total decrease in -SH which resulted from the flour treatments was equivalent to approximately 100 ppm glutathione, a quantity which is sufficient to produce marked effects in breadmaking. Changes in gluten strength did not parallel the reductions in -SH content due to oxidation.

The reasons for the effects of -SH compounds upon the baking properties of flour are not shown by these studies. Such effects may be due to some properties similar to the peptizing action of cysteine but of a much milder nature.

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THE EFFECT OF LEVEL OF SOIL FERTILITY ON WHEAT QUALITY[1]

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Plant food is being mined from all soils where crops are grown. The fertility of the soil, especially in the humid region, is rapidly declining. Wheat plants and wheat seedlings grown in this region are showing nitrogen and phosphorus deficiency symptoms, and some wheat fields even indicate potash starvation. It is essential, therefore, to investigate the influence of the level of soil fertility on the quality of the wheat crop.

Several reports have been published on the influence of various fertilizers and soil treatments on wheat composition, but few data are available on the effect of different levels of soil fertility on quality in wheat.

Working with soft winter wheats, Bayfield (1936) reported that the protein content in wheat tends to increase with increasing heaviness of texture and also with increasing fertility of the soil upon which the wheat is grown. Fisher and Jones (1931) showed that, in general, the fertilized plots produced wheat of better baking quality than the unfertilized plots. However, the order of the quality of the various plots was different every year. Results reported by Sullivan *et al* (1938) indicated that the plots which had not received any fertilizer produced wheat with shrunken grains and high protein content. When complete fertilizer was used in addition to the lime, the wheat had a lower protein content, higher kernel weight, higher test weight, and lower vitreousness than when lime alone was used.

The present study reports the quality of five varieties of wheat

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when grown on soils in which different levels of soil fertility were established by the addition of mineral fertilizers. The tests were conducted on Plainfield fine sand, Crosby silt loam, and Alford silt loam soils during the 5-year period 1937 to 1941.

Materials and Methods

Five varieties of wheat that varied in inherent quality were selected and grown at all locations. These were, in order of ascending gluten strength, American Banner, Purdue No. 1, Trumbull, Michigan Amber, and Michikof. The three soil types—Plainfield fine sand, Crosby silt loam, and Alford silt loam—are on Purdue University experimental farms located near Culver, Lafayette, and Wheatland, Indiana, respectively. On each soil type, three levels of fertility were established by the addition of mineral fertilizers as follows: Low level, none; medium level, 250 lb of 2–12–6 (percentage of N, P_2O_5, K_2O) in the fall and 100 lb of sodium nitrate in the spring; high level, 500 lb of 2–12–6 and 200 lb of sodium nitrate. On the Plainfield fine sand a 3–12–12 analysis fertilizer replaced the 2–12–6.

Each level of fertility was replicated three times and each variety was replicated 15 times on each fertility level, making a total of 225 samples at each location each year. Quality analyses were made on 3150 samples obtained in this experiment. A recent publication by Worzella (1943), reporting the yield results, gives further detail as to randomization, plot size, field plan, etc., of this experiment.

Gluten strength was determined by the wheat-meal fermentation-time test as described by Cutler and Worzella (1936). Granulation (the particle-size index of wheat meal) was determined according to the method developed by Worzella and Cutler (1939, 1943). The method reported by Binnington and Geddes (1939), with some minor modifications, was used for determining the carotinoid content of wheat. A 10-g sample of finely ground wheat meal was placed in a 4-oz bottle containing 50 ml of water-saturated butanol and allowed to stand for 16 hours with occasional shaking. Clarification was effected by filtration through No. 1 Whatman paper. The yellow pigment content of the extract was determined in a 2-cm cell, using a KWSZ photometer. Pure beta-carotene was used to prepare a series of known standards. By means of a conversion table, the percentage transmittancy readings were expressed as parts of carotene per million parts of wheat meal. Crude protein and ash determinations were made according to the methods outlined in *Cereal Laboratory Methods* (4th ed. 1941), and are reported on a 13.5% moisture basis. The test weights of the small samples of wheat were determined by the method developed by Aamodt and Torrie (1934).

Except for carotinoid pigment content, protein, and ash, analyses were made on each of the 3150 wheat samples obtained in this experiment; for these particular components of quality, 630 composite samples were employed. Analysis of variance was used to aid in the interpretation of the data.

Experimental Results

The 5 years during which these data were obtained were quite favorable for wheat growing. Winter injury did not occur to any appreciable degree in any year under consideration. Lodging was not a factor in influencing the results even on the high levels of fertility. No data were obtained on the Crosby silt loam soil in 1937 because of a severe stem rust epidemic in the Lafayette area. Except during the dry weather in April 1938 and May 1939 on the Plainfield fine sand (Culver), moisture was adequate throughout the wheat growing season each year at all locations. All varieties of wheat grown on the higher levels of soil productivity ripened from 1 to 2 days earlier than those on the low fertility plots.

The experimental data on yield, gluten strength, granulation, carotinoid pigment, crude protein, test weight, kernel size, and ash are presented in the order named. Since the experimental farms are located some 200 miles apart, factors such as climate, soil type, adaptation, etc., interfere in making a direct comparison between locations. Consequently, the data for the three locations have been summarized and analyzed separately.

Yield. Yield may be regarded as the ultimate expression of all environmental conditions and inherent factors that have integrated throughout the life of the plant. Grain yield results, therefore, aid in the interpretation of quality studies. The yield data, involving the samples used in this study, have been reported in a recent publication by Worzella (1943). It was shown in the above report that, on the basis of 4- or 5-year averages, the yields in bushels per acre for low, medium, and high levels of soil fertility were as follows: 14.2, 22.2, and 25.0 for the Sand Field; 19.7, 30.4, and 35.2 for the Knox County Experimental Farm; and 27.8, 35.0, and 40.7 for the Soils and Crops Farm, respectively. The yield data indicate, therefore, that the wheat samples used in these quality studies were grown on widely different soil productivity levels.

Gluten Strength. The data for fermentation time and analyses of variance of the five varieties of wheat grown on the three levels of soil fertility at the three locations during the 5-year period 1937–41 are reported in Table I.

TABLE I

FERMENTATION TIME FOR GLUTEN STRENGTH AND ANALYSES OF VARIANCE OF FIVE VARIETIES OF WHEAT GROWN ON THREE LEVELS OF SOIL FERTILITY AT THREE LOCATIONS DURING THE 5-YEAR PERIOD, 1937–41

Levels of soil fertility	Fermentation time in minutes					Mean
	Purdue I	Trumbull	American Banner	Michigan Amber	Michikof	
SAND FIELD (CULVER)						
Low	38.1	41.6	25.1	48.7	185.1	67.7
Medium	43.7	44.1	27.1	54.6	200.3	74.0
High	48.8	45.3	29.6	55.1	193.7	74.5
Mean	43.5	43.7	27.3	53.0	193.1	72.1
KNOX COUNTY EXPERIMENTAL FARM (WHEATLAND)						
Low	33.5	36.3	22.4	44.8	158.5	59.1
Medium	35.7	38.0	23.6	50.4	176.0	64.8
High	37.8	40.6	25.8	56.2	185.2	69.1
Mean	35.7	38.2	23.9	50.5	173.2	64.3
SOILS AND CROPS FARM (LAFAYETTE) [1]						
Low	33.4	34.1	22.3	43.2	150.5	56.7
Medium	35.0	35.0	23.0	45.0	154.4	58.5
High	36.2	37.3	24.8	47.8	170.0	63.2
Mean	34.9	35.5	23.4	45.3	158.2	59.4

ANALYSIS OF VARIANCE

Item	Degrees of freedom	Mean square		
		Sand Field	Knox County Expt. Farm	Soils and Crops Farm [1]
Season	4	1,183†	1,062†	645†
Varieties	4	69,902†	56,921†	37,300†
Fertility levels	2	357†	631†	224†
Varieties × levels	8	46	127†	59†
Seasons × varieties	16	373†	608†	687†
Seasons × levels	8	19	19*	22
Seasons × levels × varieties	32	24	8	11

[1] Includes the 4 years, 1938–41.
* Exceeds the 5% level of significance.
† Exceeds the 1% level of significance.

An examination of the fermentation-time test data shows that at any one location the gluten strength, as measured by the fermentation-time test, increased as the level of soil fertility increased. This relationship exists in the weaker quality soft wheats as well as the stronger

gluten variety Michikof. These differences are very consistent and therefore highly significant. Although higher fertility of the soil definitely increases gluten strength, the greatest variation is the inherent difference between varieties. The results of the analysis indicate, also, that great differences exist between seasons, and that varieties respond differently in different seasons.

Granulation. Granulation tests were conducted on the wheat samples to determine the influence of soil fertility on the ease of "breaking up" or disintegration of the wheat kernel by grinding—an important milling characteristic. The data, expressed in percentage and designated as particle-size index, together with analyses of variance, are given in Table II.

The data indicate that on the Sand Field plots fertility had little, if any, influence on granulation of wheat meal. However, at the Knox County Experimental Farm and the Soils and Crops Farm, wheat samples grown on the more productive levels milled into coarser meal than those produced on the lower fertility plots. These differences, although significant, are small in magnitude. It is concluded, therefore, that granulation is a highly stable varietal characteristic, and is influenced only slightly by wide variations in soil productivity. The greatest spread in granulation is the result of variety. Purdue I, a soft wheat, shows a particle index of about 20%, whereas the hard wheat, Michikof, possesses an index of about 11%. The highly significant seasons × varieties and seasons × levels interactions indicate that varieties and levels responded differently in different seasons.

Carotinoid Pigment Content. The amount of carotinoid pigment in wheat is usually reflected by color, an important characteristic of white flour. Table III reports the amount of carotinoid pigment of wheat when grown under conditions of low, medium, and high soil fertility.

The values for total carotinoid content in wheat shown in Table III are lower than those usually reported in the literature. There is disagreement among investigators as to the material best suited for the preparation of standards in the calibration of photoelectric instruments. In this study, pure beta-carotene, which is not completely soluble in water saturated butanol, was used.

It will be noted from the data that the carotinoid content of wheat decreased as the level of fertility increased. These differences are highly significant, and the data are consistent at each of the three locations. The analysis of variance indicates that the varieties reacted uniformly in respect to carotinoid content on all levels of soil fertility. Seasons × varieties and seasons × levels interactions are highly significant.

TABLE II

PARTICLE-SIZE INDEX OF WHEAT MEAL AND ANALYSES OF VARIANCE OF FIVE VARIETIES OF WHEAT GROWN ON THREE LEVELS OF SOIL FERTILITY AT THREE LOCATIONS DURING THE 5-YEAR PERIOD, 1937–41

Levels of soil fertility	Particle-size index in percent					Mean
	Purdue I	Trumbull	American Banner	Michigan Amber	Michikof	
SAND FIELD (CULVER)						
Low	22.2	19.8	18.9	18.2	11.2	18.1
Medium	21.7	18.9	18.6	18.4	11.2	17.8
High	22.8	18.5	18.4	18.2	11.3	17.8
Mean	22.2	19.1	18.7	18.3	11.2	17.9
KNOX COUNTY EXPERIMENTAL FARM (WHEATLAND)						
Low	19.2	19.5	18.3	17.3	10.9	17.0
Medium	18.7	19.0	17.7	16.6	10.8	16.6
High	18.6	19.2	17.4	16.5	11.3	16.6
Mean	18.8	19.2	17.8	16.8	11.0	16.7
SOILS AND CROPS FARM (LAFAYETTE) [1]						
Low	19.8	18.3	18.2	17.9	11.6	17.2
Medium	19.8	17.6	18.0	17.2	11.6	16.8
High	19.0	17.0	17.2	16.4	11.2	16.2
Mean	19.5	17.7	17.8	17.2	11.4	16.7

ANALYSIS OF VARIANCE

Item	Degrees of freedom	Mean square		
		Sand Field	Knox County Expt. Farm	Soils and Crops Farm [1]
Seasons	4	135.48*	48.74*	38.24*
Varieties	4	245.54*	167.18*	113.75*
Fertility levels	2	0.64	1.67*	5.06*
Varieties × levels	8	0.85	0.36*	0.25
Seasons × varieties	16	5.61*	1.99*	1.54*
Seasons × levels	8	8.47*	1.14*	3.87*
Seasons × levels × varieties	32	0.65	0.09	0.20

[1] Includes the 4 years, 1938–41.
* Exceeds the 1% level of significance.

Crude Protein. The protein content of wheat and analyses of variance data for the fertility level experiments are given in Table IV. Wheat produced on soil of the highest fertility level at each of the three locations shows the highest percentage of protein. Plots receiving no

fertilizer produced wheat somewhat higher in protein content than those receiving only a moderate amount of plant food.

Although the five varieties used in this study are known to vary widely in their milling and baking characteristics, their range in average protein content is less than 1%. The average protein content of

TABLE III

CAROTINOID PIGMENT CONTENT OF WHEAT MEAL AND ANALYSES OF VARIANCE OF FIVE VARIETIES OF WHEAT GROWN ON THREE LEVELS OF SOIL FERTILITY AT THREE LOCATIONS DURING THE 5-YEAR PERIOD, 1937–41

Levels of soil fertility	Yellow pigments (expressed as carotene) in parts per million					Mean
	Purdue I	Trumbull	American Banner	Michigan Amber	Michikof	
SAND FIELD (CULVER)						
Low	1.82	1.92	1.79	1.58	1.93	1.81
Medium	1.69	1.72	1.67	1.46	1.81	1.67
High	1.64	1.70	1.61	1.42	1.72	1.62
Mean	1.72	1.78	1.69	1.48	1.82	1.70
KNOX COUNTY EXPERIMENTAL FARM (WHEATLAND)						
Low	1.84	2.07	2.03	1.70	2.10	1.95
Medium	1.67	1.84	1.86	1.47	1.94	1.76
High	1.65	1.79	1.78	1.46	1.87	1.71
Mean	1.72	1.90	1.89	1.54	1.97	1.80
SOILS AND CROPS FARM (LAFAYETTE)[1]						
Low	1.99	2.11	2.01	1.84	2.24	2.04
Medium	1.83	1.91	1.93	1.65	2.10	1.88
High	1.81	1.90	1.89	1.62	2.07	1.86
Mean	1.88	1.97	1.94	1.70	2.14	1.93

ANALYSIS OF VARIANCE

Item	Degrees of freedom	Mean square		
		Sand Field	Knox County Expt. Farm	Soils and Crops Farm[1]
Season	4	0.7853*	2.5514*	0.3414*
Varieties	4	0.2534*	0.4469*	0.2966*
Fertility levels	2	0.2378*	0.3937*	0.1906*
Varieties × levels	8	0.0023	0.0030	0.0026
Seasons × varieties	16	0.0074*	0.0134*	0.0149*
Seasons × levels	8	0.0138*	0.0247*	0.0354*
Seasons × levels × varieties	32	0.0012	0.0025	0.0032

[1] Includes the 4 years, 1938–41.
* Exceeds the 1% level of significance.

TABLE IV

PROTEIN CONTENT OF WHEAT AND ANALYSES OF VARIANCE OF FIVE VARIETIES OF WHEAT GROWN ON THREE LEVELS OF SOIL FERTILITY AT THREE LOCATIONS DURING THE 5-YEAR PERIOD, 1937–41

Levels of soil fertility	Protein content in percent					Mean
	Purdue I	Trumbull	American Banner	Michigan Amber	Michikof	
SAND FIELD (CULVER)						
Low	10.02	11.55	10.39	10.89	11.11	10.79
Medium	10.20	11.08	10.17	10.66	10.97	10.61
High	11.23	12.45	11.11	11.77	12.22	11.76
Mean	10.48	11.69	10.56	11.11	11.43	11.05
KNOX COUNTY EXPERIMENTAL FARM (WHEATLAND)						
Low	10.14	10.96	10.22	10.56	10.98	10.57
Medium	9.74	10.62	9.98	10.26	10.52	10.22
High	10.42	11.28	10.10	10.78	11.24	10.76
Mean	10.10	10.95	10.10	10.53	10.91	10.52
SOILS AND CROPS FARM (LAFAYETTE) [1]						
Low	8.27	9.31	8.68	8.78	8.97	8.80
Medium	8.21	9.26	8.58	8.72	8.99	8.75
High	8.78	9.76	9.03	9.30	9.41	9.26
Mean	8.42	9.44	8.76	8.93	9.12	8.94

ANALYSIS OF VARIANCE

Item	Degrees of freedom	Mean square		
		Sand Field	Knox County Expt. Farm	Soils and Crops Farm [1]
Season	4	23.56*	12.56*	1.39*
Varieties	4	4.22*	2.61*	1.77*
Fertility levels	2	9.39*	1.88*	1.54*
Varieties × levels	8	0.10	0.08	0.01
Seasons × varieties	16	0.21*	0.20*	0.12*
Seasons × levels	8	2.86*	0.40*	0.06*
Seasons × levels × varieties	32	0.06	0.04	0.01

[1] Includes the 4 years, 1938–41.
* Exceeds the 1% level of significance.

the varieties studied does not reflect their known gluten strength. American Banner, a white wheat, is known to possess the weakest quality, yet its protein content is higher than that of Purdue No. 1. Trumbull, a typical soft wheat, also shows a higher percentage of

protein, on all levels of soil fertility and at all locations, than Michikof, a hard wheat. These data indicate that protein content is of little, if any, value in appraising the gluten quality of wheats grown in the soft winter wheat area. The results shown in Table IV also substantiate the well-known fact that great differences exist among seasons

TABLE V

TEST WEIGHT OF WHEAT AND ANALYSES OF VARIANCE OF FIVE VARIETIES OF WHEAT GROWN ON THREE LEVELS OF SOIL FERTILITY AT THREE LOCATIONS DURING THE 5-YEAR PERIOD, 1937–41

Levels of soil fertility	Test weight in pounds per bushel					Mean
	Purdue I	Trumbull	American Banner	Michigan Amber	Michikof	
SAND FIELD (CULVER)						
Low	58.2	59.0	57.5	58.9	59.6	58.6
Medium	58.4	59.0	57.3	58.8	59.6	58.6
High	58.3	58.9	57.6	58.8	59.5	58.6
Mean	58.3	59.0	57.5	58.8	59.6	58.6
KNOX COUNTY EXPERIMENTAL FARM (WHEATLAND)						
Low	58.5	58.4	57.0	58.5	58.7	58.2
Medium	59.5	59.0	57.6	59.2	59.6	59.0
High	59.5	59.2	57.9	59.5	59.7	59.2
Mean	59.2	58.9	57.5	59.1	59.3	58.8
SOILS AND CROPS FARM (LAFAYETTE) [1]						
Low	59.7	59.6	57.8	59.1	59.5	59.1
Medium	60.3	60.1	58.0	60.0	60.2	59.7
High	60.5	60.3	58.4	60.3	60.4	60.0
Mean	60.2	60.0	58.1	59.8	60.0	59.6

ANALYSIS OF VARIANCE

Item	Degrees of freedom	Mean square		
		Sand Field	Knox County Expt. Farm	Soils and Crops Farm [1]
Season	4	63.49*	24.67*	18.96*
Varieties	4	9.42*	8.25*	9.46*
Fertility levels	2	0.01	6.25*	3.68*
Varieties × levels	8	0.05	0.04	0.06
Seasons × varieties	16	1.05*	0.31*	0.35*
Seasons × levels	8	2.78*	0.39*	0.31*
Seasons × levels × varieties	32	0.11	0.04	0.05

[1] Includes the 4 years, 1938–41.
* Exceeds the 1% level of significance.

TABLE VI

THOUSAND-KERNEL WEIGHT OF WHEAT AND ANALYSES OF VARIANCE OF FIVE VARIETIES OF WHEAT GROWN ON THREE LEVELS OF SOIL FERTILITY AT THREE LOCATIONS DURING THE 5-YEAR PERIOD, 1937–41

Levels of soil fertility	1000-kernel weight in grams					Mean
	Purdue I	Trumbull	American Banner	Michigan Amber	Michikof	
SAND FIELD (CULVER)						
Low	26.4	30.5	32.0	27.9	26.1	28.6
Medium	28.7	33.3	34.7	30.3	28.2	31.0
High	28.5	32.9	35.0	30.3	27.9	30.9
Mean	27.9	32.2	33.9	29.5	27.4	30.2
KNOX COUNTY EXPERIMENTAL FARM (WHEATLAND)						
Low	26.6	28.4	29.0	28.1	25.0	27.4
Medium	28.7	30.5	31.5	30.2	27.4	29.7
High	28.8	30.6	32.2	30.1	27.7	29.9
Mean	28.0	29.8	30.9	29.5	26.7	29.0
SOILS AND CROPS FARM (LAFAYETTE) [1]						
Low	31.0	33.3	35.1	31.0	28.8	31.8
Medium	32.6	35.0	36.4	33.1	30.4	33.5
High	32.3	35.0	36.8	32.9	30.7	33.5
Mean	32.0	34.4	36.1	32.3	30.0	33.0

ANALYSIS OF VARIANCE

Item	Degrees of freedom	Mean square		
		Sand Field	Knox County Expt. Farm	Soils and Crops Farm [1]
Season	4	57.59†	59.48†	147.39†
Varieties	4	119.66†	40.54†	68.00†
Fertility levels	2	48.96†	46.70†	19.07†
Varieties × levels	8	0.30	0.27	0.17
Seasons × varieties	16	1.58†	1.24†	2.73†
Seasons × levels	8	1.07*	2.48†	2.22†
Seasons × levels × varieties	32	0.35	0.15	0.16

[1] Includes the 4 years, 1938–41.
* Exceeds the 5% level of significance.
† Exceeds the 1% level of significance.

and locations. Under the conditions of the experiment, all varieties reacted uniformly in protein content on all levels of soil fertility.

Test Weight. Test weight is an important component of wheat quality since it generally reflects flour yield. The data involving the

test weight of five varieties of wheat grown on several levels of soil fertility are shown in Table V.

On the Plainfield fine sand at Culver, Indiana, there was no relation between test weight and soil fertility. At the Knox County Experimental Farm the average test weight was increased from 58.2 lb on

TABLE VII

Ash Content of Wheat and Analyses of Variance of Five Varieties of Wheat Grown on Three Levels of Soil Fertility at Three Locations During the 3-year Period, 1939–41

Levels of soil fertility	Ash content in percent					Mean
	Purdue I	Trumbull	American Banner	Michigan Amber	Michikof	
SAND FIELD (CULVER)						
Low	2.06	1.96	1.86	1.95	1.89	1.94
Medium	1.95	1.87	1.79	1.82	1.81	1.85
High	1.94	1.91	1.80	1.80	1.78	1.85
Mean	1.98	1.91	1.82	1.86	1.83	1.88
KNOX COUNTY EXPERIMENTAL FARM (WHEATLAND)						
Low	1.76	1.68	1.69	1.67	1.67	1.69
Medium	1.76	1.68	1.70	1.70	1.66	1.70
High	1.79	1.78	1.70	1.72	1.70	1.74
Mean	1.77	1.71	1.70	1.70	1.68	1.71
SOILS AND CROPS FARM (LAFAYETTE)						
Low	1.90	1.89	1.83	1.87	1.79	1.85
Medium	1.84	1.86	1.79	1.81	1.74	1.81
High	1.86	1.86	1.77	1.81	1.79	1.82
Mean	1.86	1.87	1.80	1.83	1.77	1.83

Analysis of Variance

Item	Degrees of freedom	Mean square		
		Sand Field	Knox County Expt. Farm	Soils and Crops Farm
Seasons	2	0.7157†	0.0488†	0.5324†
Varieties	4	0.0436†	0.0074†	0.0160†
Fertility levels	2	0.0464†	0.0060†	0.0092†
Varieties × levels	8	0.0013	0.0009	0.0007
Seasons × varieties	8	0.0036*	0.0044*	0.0013*
Seasons × levels	4	0.0029	0.0155†	0.0007
Seasons × levels × varieties	16	0.0010	0.0011	0.0005

* Exceeds the 5% level of significance.
† Exceeds the 1% level of significance.

the low-fertility to 59.2 lb on the high-fertility plots. On the Soils and Crops Farm, the average test weights for low, medium, and high levels of soil fertility were 59.1, 59.7, and 60.0 lb, respectively. Varieties varied significantly in test weight at all locations with a range of averages from 57.7 lb for American Banner to 59.6 for Michikof.

Kernel Size. Kernel size was determined by the weight in grams of 1000 kernels of wheat. The data were averaged according to varieties and locations and are shown in Table VI.

It will be noted that the low-fertility plots at each location produced the smallest kernels, while the wheat grown on the soils of medium and high productivity had significantly larger kernels. Significant differences were also found between seasons and between varieties.

Ash Content. Ash determinations were made only on the wheat samples harvested during the 3-year period, 1939–41. The data are presented in Table VII.

At the Sand Field and the Soils and Crops Farm the wheat grown on the low-fertility plots possessed a greater percentage of ash than that produced on the more productive levels. On the other hand, at the Knox County Farm, the samples from the plots receiving the heavy application of fertilizers showed the higher percentage of ash. Little difference was found in the ash content of wheat grown on the medium and high levels of fertility at all three locations. All varieties reacted uniformly in ash content on all levels of soil fertility.

Summary

Gluten strength, granulation, carotinoid pigment, crude protein, test weight, kernel size, and ash data are reported for five varieties of wheat grown on three levels of soil fertility at each of three locations during the 5-year period of 1937–41.

The results show that soil fertility definitely influences quality in wheat. In general the quality of wheat improves as the fertility of the soil increases. Wheat produced on the well-fertilized plots was found stronger in gluten, lower in carotinoid pigments, and higher in yield of flour than that grown on the low-fertility plots. Variety, or heredity, caused the greatest variations and had the most influence in producing differences in the components of quality studied.

Since the fertility of the soil, especially in the humid region, is rapidly declining, the results would indicate that the quality of our future wheat supply in this region may be somewhat lower than that formerly obtained. However, by the addition of adequate amounts of fertilizer to the soil, wheat of satisfactory quality can be produced. Moreover, since most components of quality are hereditary, they can be amended to some extent by the breeder.

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SIMPLIFIED PROCEDURES FOR THE DETERMINATION OF THIAMINE IN WHEAT FLOURS AND BREAD BY THE THIOCHROME METHOD

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With the introduction of the enrichment program, a growing number of laboratories have been applying an increasing amount of time to thiamine assay in wheat products. The saving of the chemist's time and of materials is always a worthy end, but it is even more imperative today that analytical procedures should require the least possible time consistent with the necessary accuracy, and that a minimum of laboratory materials be employed.

A shortened procedure for the thiochrome method was reported by Andrews and Nordgren (1941) who employed a 15-min extraction at room temperature with 25% potassium chloride in 2% acetic acid, followed by filtration and oxidation to thiochrome. The oxidation was

performed according to the method of the American Association of Cereal Chemists (Cereal Laboratory Methods, 4th ed. 1941).

Hoffer, Alcock, and Geddes (1943) have just published a modification of the procedure of Andrews and Nordgren (1941) in which the extraction is carried out at 70° for 30 min. This method, when compared with that of the A.A.C.C., was shown to give low results with unenriched commercial flours, but the difficulty could be circumvented by the use of a correction factor. For wheat and enriched flour there was no need for the correction.

Meanwhile, in England, an abbreviated procedure was independently developed by Nicholls, Booth, Kent-Jones, Amos, and Ward (1942) and by Booth (1942) for the estimation of thiamine in the national wheat meal. They omitted hot extraction, enzyme digestion, and base exchange purification. However, their procedure differed from that of the above American workers in the use of mineral acid without salt for the extraction and in the introduction of methanol prior to oxidation, as recommended by Harris and Wang (1941) and Wang and Harris (1942) for the stabilization of thiochrome.[1] A comparison of this method, with a few minor modifications, with the collaborative method of Hennessy (1942), changed in only one detail, was undertaken for wheat flours and bread. The Hennessy method was chosen for the comparison since, in the opinion of the author, it is the best of the American methods employing the adsorption step. Consideration was also given to the question of the stability of standard solutions of thiamine.

Experimental

Simplified Procedure for Patent, Straight, Clear, Low Grade, and Enriched Flours.

A guide to the proportion of extracting acid to sample weight has been given by Hennessy (1942). We have found it convenient to employ 50 ml of 0.1*N* H_2SO_4 to 10 g of patent or straight, 4 g of enriched patent or straight, 8 g of clear, or 2 g of low grade flour.

Add the sample to the acid in a graduate with a ground glass mouth, stopper, and place in a mechanical rocker or slow shaker for 30 min or shake by hand at least once every 5 min. Filter through a paper having the porosity of No. 4 Whatman, fluted to increase the filtration rate, and after the filtrate begins to come through clear, pour back the first turbid liquid for a second filtration. In each of two separatory centrifuge tubes (E. Machlett & Sons) mix 5 ml of extract with 5 ml CH_3OH. To one tube add rapidly 3 ml of 15% NaOH, from a pipette with a large orifice, followed immediately by 2 drops of 1% $K_3Fe(CN)_6$; mix at once, and after 45–60 seconds add 20 ml of isobutanol previously saturated with distilled water. Repeat with the other tube but omit the $K_3Fe(CN)_6$. Stopper and shake tubes well for about 60 seconds. Centrifuge at 500–600 rpm for 30–45 seconds and drain off the bottom layer. To remove the water still suspended in the isobutanol solution, filter

[1] Since this manuscript was submitted, a report has been published by the Vitamin B_1 Subcommittee of the Accessory Food Factors Committee of the Medical Research Council and the Lister Institute, giving the results of comparative tests for thiamine by various methods (Biochem. J. **37**: 433–439. 1943).

through No. 4 Whatman or similar grade of fast paper. Pass the filtrate through a fresh paper in a clean funnel directly into the tube or cuvette employed with the fluorometer. Measure the fluorescence of the unknown (X) and the blank (B_x) after adjusting the instrument to maximum galvanometer scale reading with a standard quinine sulfate solution (for the Pfaltz and Bauer instrument we use 0.5 μg per ml in $0.1N$ H_2SO_4). Standardization with a thiamine solution of known concentration is effected by placing 5 ml of a standard solution of thiamine hydrochloride in $0.1N$ H_2SO_4 (0.4 μg of thiamine per ml) into each of two separatory centrifuge tubes and treating in the same manner as the extract to obtain the fluorescence of the standard (S) and the blank (B_s).

The data are used to calculate the concentration of thiamine in the sample as follows:

$$\mu\text{g thiamine per g flour} = \frac{X - B_x}{S - B_s} \cdot \frac{50}{W} \cdot \frac{2}{5}$$

where W = weight of sample in grams.

Procedure for Whole Wheat Flour. Since whole wheat contains significant concentrations of interfering substances that decrease fluorescence, it is necessary to eliminate their effect. This may be accomplished by measurement of the fluorescence produced by the extract in the presence and absence of added thiamine as follows:

To 50 ml of 2% HCl (wt./vol.) in a graduate, add 4 g of the sample, stopper, shake, and allow to stand overnight at room temperature. In a separate graduate, repeat in a parallel manner with 2% HCl containing 8 μg of thiamine hydrochloride (2 μg/g sample). In the morning, filter as in the preceding section. Use 5 ml of filtrate in each case for the oxidation to thiochrome, following the procedure outlined previously except that 2 drops of 3% $K_3Fe(CN)_6$ are used. Measure the fluorescence produced by the extract (X), and by the extract containing the added thiamine (S). Blank determinations are carried out by omitting the $K_3Fe(CN)_6$ as previously described. The calculation is:

$$\mu\text{g thiamine per g sample} = (X - B_x)\left(\frac{2}{(S - B_s) - (X - B_x)}\right)$$

Since B_x and B_s in this case are essentially equal,

$$\mu\text{g per g} = \frac{X - B_x}{S - X} \cdot 2$$

Procedure for Bread.

To 50 ml of acetate buffer having a pH of 4.0 (36 ml of $0.1M$ CH_3COONa + 164 ml of $0.1M$ CH_3COOH) add 8 g of sample and 0.2 g of takadiastase. Another vessel is prepared in the same way except that the acetate buffer contains 16 μg of thiamine hydrochloride. After shaking well, the vessels are kept at 40°C overnight. The filtration of the extract and subsequent steps follow exactly the procedure given for whole wheat, and the calculation is identical.

Comparison of Methods

The flours used to obtain the comparative data in Table I were milled from a wide variety of spring and winter wheats grown in various localities, and the data demonstrate the satisfactory agreement between assays made according to the procedures which have just been described and a slight modification of the collaborative method of Hennessy (1942). The departures from the Hennessy method consisted of adding the alkali before the ferricyanide in the oxidation step,

TABLE I

COMPARISON OF THIAMINE METHODS

Sample	Hennessy method[1]	Present short method
	μg/g	μg/g
Flours		
Enriched patents	4.40	4.40
	4.58	4.54
	4.78	4.76
	4.58	4.58
	4.67	4.62
Patent	0.56	0.55
	0.59	0.59
	0.38	0.38
	0.38	0.38
	0.37	0.38
Straight grade	0.80	0.80
	0.95	0.94
	1.10	1.09
	0.58	0.62
	0.89	0.83
Clear	2.49	2.40
	2.04	2.05
	2.20	2.35
	2.11	2.15
	1.40	1.30
Low grade	7.07	6.73
	6.99	7.36
	9.25	9.44
	5.67	5.39
	2.83	2.93
Whole wheat	5.00	5.07
	5.30	5.33
	3.86	3.76
	4.75	4.75
	3.82	4.07
Bread		
White bread[2]	2.28	2.42
	2.28	2.27
	2.16	1.98
	2.55	2.64
	2.64	2.46

[1] These data have been corrected for the volume of the sample in the extracting medium; furthermore the alkali was added before the ferricyanide in these determinations.
[2] Values converted to 38% moisture basis.

and in correcting the results for the volume of the sample in the extracting medium. In the published method no such correction is made, and consequently the results are always too high. For samples under 4 g the magnitude of the correction is small and usually may be neglected, but it was found that with a 10-g sample an error of about 7.0% is involved and a 5-g sample gave an error of about 3.5%. This

was shown in the following manner: The 75 ml of acid plus sample plus 5 ml of acetate-enzyme solution required 13 ml of water in the case of a 10-g sample, a total of 93 ml of liquid to make up the volume to the 100-ml mark on a volumetric flask; for a 5-g sample the total volume of liquid added was 96.5 ml. However, the calculation is based on the presence of 100 ml of liquid which is obviously not there. It would be simpler, of course, to employ a fixed volume of liquid and thus obviate the need for a correction. This is the procedure followed in both the British and regular A.A.C.C. methods.

Notes

There are a number of points concerning the foregoing procedures that deserve mention:

The principle of standardizing a determination by reference to a parallel measurement on an equivalent sample to which a known quantity of thiamine has been added is the soundest to employ. In this manner, all of the interfering influences that might be present exert equal effects on both the determination and the standardization and thus are cancelled. Since each sample must be standardized separately, this method involves more measurements than in the usual procedure of applying one control estimation on a standard thiamine solution to all of the determinations made at one time. For those flours which are relatively free from interfering concomitant materials, the latter method can be safely employed, but for whole wheat flours or bread this is not the case; hence the difference in the standardization procedure given for the latter compared to that for the patents, clears, etc. For instance, a whole wheat flour giving a value of 5.07 μg/g by the procedure described in the present paper gave only 3.74 μg/g when standardized against a pure thiamine solution subjected to the same treatment.

It is of interest to note that the volume of extracting acid does not enter into the calculation for the whole wheat and bread methods. Provided the concentration of thiamine in the extract is in the proper range, the actual volume of acid used will be without influence as long as an equal volume is employed in the standardization.

In those instances where the 30-min extraction period is used, the subsequent filtration requires an additional 30 min or so, depending on the nature of the flour and the size of the sample. The true extraction period is thus greater than 30 min. With more coarsely ground material, the extraction time would naturally have to be longer, and that is the basis for the overnight extraction of the whole wheat flour. This period is probably greater than necessary but was employed for convenience. A sample giving a thiamine content of 5.07 μg/g,

when subjected to overnight extraction, showed only 3.72 $\mu g/g$ when extracted for 30 min.

With flours which are milled from wheat mixes containing appreciable percentages of Durum wheat, a serious interfering fluorescence can be eliminated, as recommended by Harris and Wang (1941) and Booth (1942), by first shaking the acid extract with isobutanol, separating, and discarding the isobutanol layer before adding the methanol and reagents for oxidation.

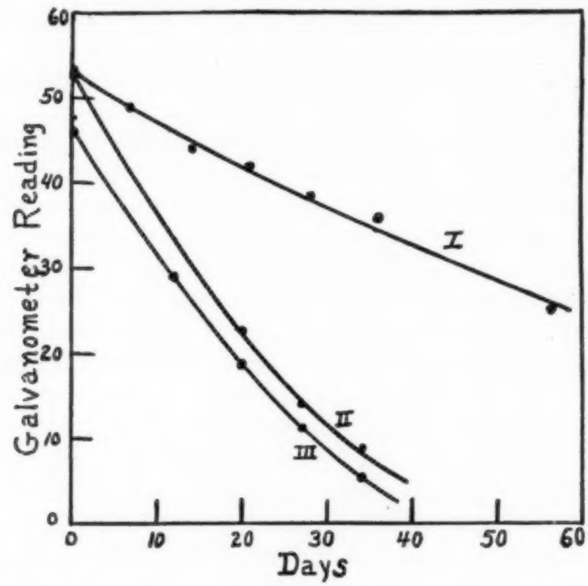

Fig. 1. Stability of standard thiamine solutions (0.4 $\mu g/ml$) stored in the dark at 6°. I. In $0.1N$ H_2SO_4 (5 ml used with 5 ml CH_3OH prior to oxidation); II. In a solution of equal volumes of $0.1N$ H_2SO_4 and CH_3OH (5 ml used with 5 ml, $CH_3OH - 0.1N$ H_2SO_4, 1 : 1); III. In absolute CH_3OH (5 ml used with 5 ml $0.1N$ H_2SO_4).

Wang and Harris (1942) have indicated the value of adding the alkali before the ferricyanide in the oxidation step. In the procedures of Hennessy (1942) and Hoffer *et al* (1943) the two are added together, and in the A.A.C.C. method they are added in the reverse order.

The amount of ferricyanide required will depend on the nature and quantity of the oxidizable impurities present in the extract. Wang and Harris (1942) have indicated that the proper amount is the least necessary to maintain the yellow ferricyanide color for over 30 seconds.

The use of either filtration through paper or addition of ethanol to clarify the isobutanol solution of thiochrome (Nicholls *et al,* 1942) is simpler, in the author's opinion, than the use of anhydrous sodium sulfate. The advantage of filtration over the use of ethanol for clarifi-

cation is that particles that may be suspended in the liquid are removed. We have found it unnecessary to subject Whatman No. 4 paper to preliminary extraction with isobutanol although the British workers have found this treatment advisable to remove fluorescent materials from the paper they employed.

The larger volume of isobutanol was employed to save time by discarding the last few ml in each of the two filtrations prior to the measurement of fluorescence.

Stability of Standard Thiamine Solutions

The effect of methanol on the stability of standard thiamine solutions stored in the dark at 6°C is apparent from Figure 1. While methanol stabilizes thiochrome or its formation from thiamine, as shown by Harris and Wang (1941), it increases the rate of gradual decomposition of stored thiamine solutions. Results shown in Figure 1 also indicate that it is unsafe to use a standard solution of thiamine in dilute acid after it has been stored for more than a few days.

Summary

Comparisons have been made between British procedures for the thiochrome determination of thiamine, with minor modifications, and the collaborative method of Hennessy, changed in one detail, when applied to wheat flours and bread. The two methods give results which are in satisfactory agreement, provided that a correction is applied to the latter to compensate for the volume of the sample in the extracting liquid.

Details have been reported of procedures for wheat flours that do not require extraction at elevated temperatures, enzyme digestion, or base exchange purification; and that eliminate the hot extraction and base exchange step in the case of bread. These methods are essentially the same as those of the British, and they offer certain advantages over the procedures generally used in America. Discussion of certain of the steps in the methodology has been included.

It was shown that methanol increases the rate of destruction of thiamine in solution.

Acknowledgment

The author wishes to express his appreciation to Betty Sullivan and Marjorie A. Howe for their helpful suggestions.

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FACTORS THAT INFLUENCE THE PHYSICAL AND OTHER PROPERTIES OF WHEAT. V. EFFECT OF FREQUENT RAINS ACCOMPANIED BY STORMS ON BLACKHULL, CHIEFKAN, AND TENMARQ [1]

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Extensive investigations of the effects of weathering on the quality of wheat have been made by the author (1936, 1941, 1942, 1943, 1943a, 1943b, and with Johnson, 1943), by Bracken and Bailey (1928), Whitcomb and Johnson (1928), and by Larmour, Malloch, and Geddes (1933). The results of various minor studies have also been briefly described in the annual reports of the Grain Research Laboratory, Board of Grain Commissioners for Canada, Winnipeg, for the years 1928, 1931 to 1935, 1941, and 1942. The general conclusion has been that effects of weathering on wheat, provided that no germination has occurred, are by no means as serious as indicated by a superficial visual examination of the samples.

Experimental investigations under natural conditions are difficult to make because they must be confined to seasons when the weather is suitable. This condition occurred during 1942 in Kansas where heavy rains occurred during the late part of the growing season and at harvest time. The results of tests on samples collected from the experimental fields at Manhattan and elsewhere throughout Kansas are reported in this paper.

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[1] Contribution No. 102, Department of Milling Industry.

Weather Conditions Prevailing in 1942

The harvest season of 1942 had abnormally heavy rains accompanied by storms during the greater part of June, both during ripening of wheat and the time it was mature for cutting. July had considerably less than half the normal rainfall for that month, while the August rainfall was nearer normal. Detailed rainfall during June, July, and August at Manhattan, Kansas, is given in Table I. The 12.72 inches

TABLE I

RAINFALL AT MANHATTAN, KANSAS, FOR JUNE, JULY, AUGUST, 1942

Date	June	July	Aug.	Date	June	July	Aug.
	inches	*inches*	*inches*		*inches*	*inches*	*inches*
1	2.24[1]			17	0.45		
2				18	1.20		
3		0.46	0.51	19	3.02	0.01	
4			0.06	20	1.77	0.04	
5	0.02	0.14		21		0.07	
6			0.10	22			
7	0.31		0.14	23			
8	0.01	0.48	0.02	24	1.80	0.06	
9	0.09		0.32	25		0.21	0.18
10	0.30			26			1.20
11	0.05		0.06	27			
12	0.45			28			
13	0.04		0.28	29	0.82		
14			0.40	30			
15	0.15			31			
16							
				Total	12.72	1.47	3.27

[1] On May 31 there was a rainfall of 0.46 inch.

rainfall in June at Manhattan was nearly three times normal. During the frequent rains in the middle of June, several storms occurred, causing first lodging and then twisting. This made gathering of samples difficult. Lodging occurred earlier in spots, but finally all the wheat was lodged or the straws broken and tangled.

Weather conditions during the harvest of 1942 at Manhattan were much different from the harvest of 1941 when only five light rains, totaling 0.98 inch, fell during the period from June 10 to July 22, and there were no storms in 1941. The results of the 1941 harvesting studies have been reported by Swanson (1943b).

General Plan of Investigations

Materials. Three varieties were included in the 1942 experiments —Blackhull, Chiefkan, and Tenmarq. These had been grown on plots in the field used for variety testing by the Department of Agronomy.

Plan of Studies. There were five parts to these studies: (1) changes in the wheat kernels during maturing or ripening from the early-milk

stage of the kernels to the hard-dough stage or maturity, and also the effects of drying in sun and shade; (2) effects of exposure in the field for about 4 weeks after maturity; (3) effects of exposing small shocks of wheat cut at the hard-dough stage; (4) effects of soaking wheat in the straw and wetting as grain; (5) observations on samples of the three varieties grown in various parts of Kansas.[2]

Laboratory Tests. The effects of the various factors on the wheat grain were measured by test weight, internal texture counts, and by milling and baking the samples included under parts 2 and 3 of the plan. Supplementary tests were also made for moisture content of wheat, and for protein, ash, and moisture content of flours used for baking. The samples cut before the hard-dough stage were scoured in a barley pearler.

Procedure and Results of Physical Tests on Manhattan Samples

Samples Cut before the Hard-Dough Stage

Procedure. First cutting of small samples before the hard-dough stage was on June 12. Cuttings were then made on June 15, 17, and 19. The weather was cool, cloudy and, as can be seen from Table I, rains were of almost daily occurrence up to June 20 when the wheat was approaching hard-dough stage. No brown color had appeared on the kernels harvested on June 12 and 15, but on June 17 the kernels were turning brown, and on June 19 they were nearly all brown and the interior was at the hard-dough stage. All kernels harvested before the hard-dough stage became dark brown and hard on drying. Three small bundles of each variety were cut on each date. One was used for determining the moisture content of the wheat kernels, another was placed to dry out of doors, and the remaining bundle was dried in the wheat nursery shed. The study of the effects of drying out of doors and inside was only partially satisfactory because of the prevalence of cool, cloudy weather.

The bundles used for the moisture determination of the grains were threshed on the small nursery thresher, and weighed samples of the grain were exposed to the air in shallow pans in the laboratory to obtain data for the amount of moisture lost in air drying. From the additional moisture contents subsequently obtained by the usual oven treatment of the air-dry samples, the total moisture contents at the time of cutting were calculated.

The bundles dried out of doors and bundles dried under cover were later threshed on the nursery thresher. The following determinations were made on the samples: test weight (micro method, Swanson, 1942),

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[2] These samples were made available by Dr. H. H. Laude, of the Department of Agronomy.

internal texture counts, and effects of miniature scouring on test weight. This scouring was done by passing 100-g samples through a barley pearler with the outlet gate open. Several preliminary tests had shown that this treatment was closely equivalent to scouring. The internal textures were determined on kernel sections made with a barley cutter. The data from these various measurements on wheat cut before the hard-dough stage are given in Table II.

TABLE II

MOISTURE, TEST WEIGHT, AND INTERNAL TEXTURE COUNTS OF WHEAT SAMPLES CUT BEFORE THE HARD-DOUGH STAGE

Date cut June	Moisture average all var.	Test weight								
		Blackhull			Chiefkan			Tenmarq		
		Shade	Sun	After scouring	Shade	Sun	After scouring	Shade	Sun	After scouring
	%	lb/bu	lb/bu	lb/bu	lb/bu	lb/bu	lb/bu	lb/bu	lb/bu	lb/bu
12	49	55.6	55.2	58.4	58.5	57.2	61.1	54.8	56.5	57.9
15	48	56.4	55.5	59.6	59.9	57.7	63.6	55.8	54.3	58.5
17	47	56.9	55.1	60.1	59.1	59.2	61.3	57.2	57.8	60.2
19	44	56.6	55.7	59.5	60.8	59.7	62.9	59.0	58.1	61.4
		Internal texture counts (Average for sun- and shade-dried samples)								
		Vit.	Semi vit.	Mealy	Vit.	Semi vit.	Mealy	Vit.	Semi vit.	Mealy
		%	%	%	%	%	%	%	%	%
12	49	55	35	10	94	4	2	85	12	3
15	48	65	27	8	98	2	0	93	6	1
17	47	69	24	7	97	2	1	88	7	5
19	44	64	28	8	97	2	1	76	17	7

Results. A comparison of the results for the samples cut June 12 with those cut on June 19 shows that Blackhull had made the least increase in test weight, Tenmarq the greatest increase, and Chiefkan intermediate. The comparatively small increases in test weight between June 12 and June 19 were probably due to the sometimes cloudy and rainy weather. All samples, except two of Tenmarq, decreased in test weight from drying out of doors because of shriveling. Drying out of doors would no doubt have had greater effect in hot, dry weather. The two exceptions of Tenmarq were probably due to variations in field sampling.

The test weights after scouring were in all cases higher than before this treatment, showing that the comparative looseness of the outer bran, even of immature grain, has a large influence on test weights.

While this method of scouring may not be very accurate, the figures are comparable and indicate that this treatment had the greatest effect on Blackhull and the least on Tenmarq with Chiefkan intermediate.

As to internal texture condition, Blackhull showed the lowest vitreous and highest mealy counts; Chiefkan showed the highest vitreous and lowest mealy counts; Tenmarq was intermediate. There was no consistent trend between the first and succeeding cuttings.

Samples Cut in and after Hard-Dough Stage and Exposed in Shocks

Procedure. The first cutting in this series was made on June 22, when the grain was in the hard-dough to hard stage. The weather at this time was still cool for the season. On this date and at each subsequent cutting, three bundles were gathered in order to have enough grain for milling and flour for baking. Besides these three bundles, a small sample was cut from each variety for the determination of moisture, proceeding in the same manner as with the samples cut earlier.

The wheat cut on June 22 appeared as mature as is usual when cutting is started with a binder for drying in shocks. Because of the rains and storms, the wheat was not only lodged but tangled. This made gathering difficult and the field losses increased progressively with the later cuttings. All bundles were placed in the shed soon after gathering and threshed when dry.

The bundles for exposure in the shocks were cut June 25–27 when the moisture content of the grain was about 20%. The small shocks were tied to wooden stakes and were not covered except with screen wire to keep off the birds. There were five small shocks of each variety, four of which were threshed at weekly intervals, but the last one was exposed until September 2, over 2 months. Hence, it was exposed to the heavier rains in August.

Results. Test weights were taken by the official method and milling was done on the Buhler mill. The flours were analyzed for moisture, protein, and ash. The figures for moisture and protein were used to calculate the absorption for making the mixograms (Swanson and Johnson, 1943), and also for baking. The dates of cutting the wheat and threshing the shocks, the moisture percentages of the grain at the time of cutting, the test weights, the flour yields, and ash are all given in Table III.

Highest test weights were obtained on the samples cut on June 22. After this there was a gradual decrease which was related to the time of exposure in the field. The total decrease from exposure in the field was 2.3 lb for Blackhull, 3.0 for Chiefkan, and 2.3 for Tenmarq. While Chiefkan had a higher test weight throughout than either of

TABLE III

Test Weight, Flour Yield, and Flour Ash Content for Wheat Samples Cut in and after Hard-Dough Stage and after Exposure in the Field or in Shocks

Date cut	Moisture when cut	Test weights			Flour yield and flour ash					
		Black-hull	Chief-kan	Ten-marq	Blackhull		Chiefkan		Tenmarq	
					Yield	Ash	Yield	Ash	Yield	Ash
EXPOSED IN THE FIELD										
	%	lb	lb	lb	%	%	%	%	%	%
June 22	37	57.6	60.7	58.0	72.0	0.47	72.0	0.46	71.0	0.45
26	20	56.4	59.9	53.7	71.0	0.46	73.0	0.47	72.6	0.50
July 1	13	57.0	59.1	56.2	71.0	0.46	73.0	0.45	71.1	0.43
6	14	56.3	58.4	55.8	74.0	0.46	74.0	0.49	73.2	0.45
13	11	56.7	58.2	55.8	70.9	0.44	76.0	0.46	73.4	0.46
20	12	56.0	58.0	54.6	72.8	0.41	73.7	0.45	72.2	0.44
22	12	55.3	57.7	55.7	72.0	0.44	72.0	0.48	72.0	0.45
EXPOSED IN SHOCKS										
Date threshed	Moisture when threshed									
	%	lb	lb	lb	%	%	%	%	%	%
July 1	12	56.5	59.4	52.6	70.2	0.46	72.5	0.51	71.0	0.41
6	11	55.5	59.2	56.6	70.2	0.45	72.0	0.47	71.4	0.45
13	10	54.4	59.9	55.5	70.1	0.46	71.0	0.47	73.6	0.48
20	10	55.8	59.5	55.6	72.7	0.47	72.0	0.46	71.9	0.45
Sept. 2	13	54.8	57.6	54.6	73.0	0.44	73.5	0.46	71.3	0.45

the other two, the decrease in the test weight of Chiefkan was somewhat greater.

Exposure in the shocks caused notably less change in test weight than exposure in the field. The few rains in July caused no consistent change in test weight, but the exposure to the heavier rains in August decreased the test weight of Blackhull and Tenmarq 1 lb and of Chiefkan 1.9 lb below the sample threshed July 20.

The flour yields [3] did not decrease correspondingly with the lowering of test weight, thus confirming previous investigations (Swanson, 1941, 1943b). The extraction percentages correspond to that obtained for straight flour and the ash figures indicate fairly uniform milling. The flour yields for Blackhull were significantly lower than for either Tenmarq or Chiefkan.

The changes in internal texture of the samples from the field exposure and the shock exposure are given in Table IV. The 0.82-inch

[3] Credit is due Warren F. Keller, Research Miller, for making the milling tests.

TABLE IV

Changes in Internal Texture of Wheat from Field and Shock Exposure

Date cut	Blackhull			Chiefkan			Tenmarq		
	Vit.	Semi vit.	Mealy	Vit.	Semi vit.	Mealy	Vit.	Semi vit.	Mealy
SAMPLES FROM FIELD EXPOSURE									
	%	%	%	%	%	%	%	%	%
June 22	55	30	15	98	1	1	93	5	2
26	50	36	14	98	1	1	92	5	3
July 1	44	38	18	94	6	0	62	36	2
6	25	52	23	85	8	7	21	50	29
13	12	49	39	89	9	2	27	50	23
20	5	49	46	73	26	1	25	53	22
22	17	45	38	62	27	11	14	69	17
SAMPLES FROM SHOCK EXPOSURE									
Date threshed	%	%	%	%	%	%	%	%	%
July 1	51	39	10	100	0	0	41	41	18
6	57	29	14	98	1	1	85	11	4
13	56	35	9	94	2	4	70	24	6
20	47	32	21	96	3	1	63	28	9
Sept. 2	18	46	36	87	7	6	38	25	17

rain on June 29 and the 0.46-inch rain on July 3 (Table I) seem to have produced marked changes in the internal texture since there is a notable increase in the mealy condition between the samples obtained on June 26 and July 1 and much more between the samples obtained on July 1 and July 6. Blackhull showed the greatest change and Chiefkan the least with Tenmarq intermediate. Chiefkan exhibited a remarkable resistance to exposure to rains. There were no extensive internal changes in the samples exposed in the shocks except the ones threshed September 2, indicating that, although the shocks were not covered, the compact massing of the wheat heads afforded a fairly good protection against the rains. Only the shocks left out of doors until September 2 had the dark gray appearance associated with prolonged exposure.

A comparison of the flour yields in Table III with the internal textures in Table IV shows that changes from vitreous to mealy interiors were not reflected in decreased flour yields.

Effects of Wetting Grain before and after Threshing

Observations made in previous experiments indicated that wetting wheat in the head did not produce as great changes as when water was added to the threshed grain. The glumes seemed to afford a considerable protection against wetting.

Procedure. Two bundles of each of the three varieties—Blackhull, Chiefkan, and Tenmarq—were cut on June 22 and again on June 26, when the wheat was hard and had not yet been affected by wetting by rain as shown in Table IV. After drying in the shed for a few days, one bundle from each pair was soaked for 3 hours, heads down, in a large can. This was done toward evening in order to prolong the period of wetness. The three bundles so wetted were then exposed until dry, but were protected from further wetting by rain, after which they were threshed.

The other bundle from each pair was threshed as soon as dry and the grain divided into two portions. One was used as a check, not wetted; the other portion was wetted by soaking the wheat in water for 2½ hours and then dried. The grain was intentionally soaked a somewhat shorter time than the heads. When again dry these three portions of grain were then tested for test weight (micro method) and internal textures.

TABLE V

EFFECT OF WETTING THRESHED AND UNTHRESHED WHEAT ON TEST WEIGHT AND INTERNAL TEXTURE OF THE GRAIN

Date cut	Variety	Treatment	Test weight	Internal texture		
				Vit.	Semi vit.	Mealy
			lb/bu	%	%	%
June 22	Blackhull	Not wetted	59.9	58	33	9
		Wetted in heads	56.1	46	41	13
		Wetted as grain	56.5	0	34	66
	Chiefkan	Not wetted	59.0	93	4	3
		Wetted in heads	59.5	94	3	3
		Wetted as grain	55.3	62	32	6
	Tenmarq	Not wetted	58.5	90	6	4
		Wetted in heads	56.5	58	36	6
		Wetted as grain	53.9	5	50	45
June 26	Blackhull	Not wetted	56.8	59	23	18
		Wetted in heads	56.5	38	39	23
		Wetted as grain	53.7	8	28	64
	Chiefkan	Not wetted	60.4	98	2	0
		Wetted in heads	59.2	94	5	1
		Wetted as grain	57.2	67	26	7
	Tenmarq	Not wetted	56.4	69	23	8
		Wetted in heads	54.9	61	32	7
		Wetted as grain	53.1	15	39	46

Results. The data in Table V show that wetting by soaking the heads in water notably decreased the test weight, but not as much as

by soaking the grain. The internal textures were also notably changed by soaking the heads but not nearly as much as by soaking the grain. In a previous experiment (Swanson, 1936) it was observed that soaking for 10 to 30 minutes had comparatively little effect. The artificial sprinkling of standing wheat produced no measurable effects. It is thought that the rains which fall as drizzles and at night have a much greater effect than small rains in the day time.

Results of Protein, Mixing, and Baking Tests on Manhattan Samples

Method. The flours from the wheat samples represented by the data in Tables II and IV were baked [4] using the formula: flour 100 g,

TABLE VI

PROTEIN CONTENT, MIXING TIME, AND LOAF VOLUME FOR FLOURS MILLED FROM WHEATS CUT AT THE HARD-DOUGH STAGE AND AFTER EXPOSURE TO RAINS

Date cut	Rainfall after cutting	Blackhull			Chiefkan			Tenmarq		
		Protein [1]	Mixing time	Loaf volume	Protein [1]	Mixing time	Loaf volume	Protein [1]	Mixing time	Loaf volume
EXPOSED IN THE FIELD										
	inches	%	*min*	*cc*	%	*min*	*cc*	%	*min*	*cc*
June 22	0.0	14.2	2.2	1043	15.0	1.6	860	13.7	3.1	1025
26	1.80	14.9	2.1	1060	14.9	1.6	843	15.0	3.1	1170
July 1	2.62	14.8	2.2	1023	15.2	1.7	800	14.2	3.0	1043
6	3.22	14.5	2.4	1020	15.0	1.8	870	13.5	3.1	958
13	3.70	14.1	2.2	983	15.4	1.8	840	14.3	3.3	1038
20	3.75	14.4	2.4	1008	14.5	1.8	765	14.4	3.4	1040
22	3.82	14.5	2.7	1080	14.7	1.8	825	13.9	3.6	980
EXPOSED IN SHOCKS										
Date threshed	Rainfall after threshing									
	inches	%	*min*	*cc*	%	*min*	*cc*	%	*min*	*cc*
July 1	0.0	15.2	2.2	1025	14.8	1.5	750	13.6	3.6	1010
6	0.60	14.8	2.2	1010	14.8	1.6	758	14.3	3.1	1058
13	1.08	15.2	2.2	1035	14.7	1.8	765	14.9	3.4	1055
20	1.13	13.7	2.2	950	14.9	1.6	785	14.6	3.6	1040
Sept. 2	3.61	15.0	2.4	1095	15.0	1.9	908	14.4	3.6	1028

[1] Protein content ($N \times 5.7$) is expressed on "as is" moisture basis.

dry milk solids 4 g, shortening 3 g, sugar 6 g, yeast 2 g, salt 1.5 g, $KBrO_3$ 3 mg, and water, calculated on the basis of protein and moisture. This "rich" formula had been shown in previous experiments (Swanson, 1943) to be best when observations are being made to discover

[4] Credit is due to John A. Johnson, Assistant Baking Technologist, for performing the baking tests.

any differentiation among samples exposed to varying amounts of weathering. The mixing time was calculated from the characteristics of the mixograms and a factor correlating with the bakery mixer.

Results. The loaf volume, mixing time, and protein percentages of the flours are given in Table VI. The data on grain texture and crumb color are not included since they did not seem to add any important information.

It is very evident that the loaf volumes vary most between the varieties and very little according to exposure. That is, the exposure in the field and shocks did not have any considerable effect on the baking value. The mixing time was slightly longer for the samples exposed the most; in previous investigations (Swanson, 1943b) it was found that the mixing times of the weathered samples were distinctly longer than the nonweathered.

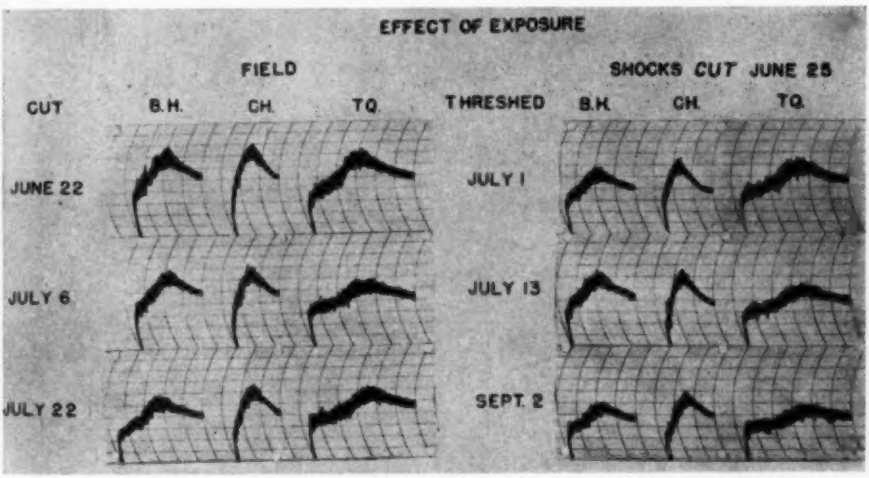

Fig. 1. Mixogram patterns for flours milled from wheat subjected to exposure in the field and in shocks.

The effects of exposure on selected mixograms are shown in Figure 1. It will be observed that some changes occur in the shapes of the curves with changes in the dates of cutting and threshing. It will be noted that the curves for Chiefkan show less change with time of exposure than do those for Blackhull and Tenmarq.

Samples from Experimental Fields in Various Parts of Kansas

The unusually wet weather which prevailed at Manhattan in June (Table I) was also experienced in the main wheat belt of Kansas. This presented an opportunity to observe the effects on these same three varieties of frequent rains between heading and harvesting in various locations.

TABLE VII

RAINFALL IN INCHES DURING KERNEL FORMATION AT VARIOUS LOCATIONS IN KANSAS

Date	Colby	Columbus	Dodge	Garden City	Hays	Hutchinson	Kingman	Meade	Thayer	Tribune
May 21										
22										
23						—[1]				
24										
25			—				—			
26										
27								—		
28		—			—					—
29										
30				—						
31			0.06	0.16	4.14		0.24	0.90		
June 1					0.25	0.50				
2	—									
3										
4	T		0.40	0.02				0.03		
5	T			T	0.04	2.00			—	
6			0.36						2.31	T
7	0.02	1.83	0.15	0.33		1.07	0.66	0.63	1.73	0.14
8	1.93	0.43	0.61	1.13	0.32	0.18	0.23	0.25	0.38	0.53
9	0.40	1.25		0.01	0.74	0.10	0.06	2.05	0.64	2.51
10		0.73				0.03		0.11		
11		0.63								
12	0.05			0.15	0.41	0.75	0.40	0.06	0.83	
13	0.62	0.97			0.15					0.72
14				0.18	T		0.09			
15		0.42			0.22	0.13			0.68	0.15
16	0.07			T	0.39	1.17	0.27			
17	0.02	0.49	0.84	0.22	2.37	1.78	0.54		1.21	
18	0.59			0.06	0.91	0.79		1.88	1.73	0.03
19		1.09			T	0.51	1.21		0.73	T
20	0.05	=[1]		0.13					1.05	
21	0.10		0.56	0.90	0.08	0.90	1.03		1.70	0.16
22	0.30		=	0.01	0.33	0.40				1.11
23	0.02			0.03	T				0.33	0.10
24	T	0.62								0.06
25	0.02									
26	T									
27				T	0.79	=				
28				0.17	0.09	0.61				
29						0.54	2.01	=		0.55
30				=	=				=	0.12
July 1	T			0.03						0.07
2				0.09						0.02
3	=									
4							=			
5										=
Total	4.19	7.84	2.98	3.50	11.45	10.31	6.74	5.91	13.32	6.27

[1] Single line represents date of heading and double line date of cutting.

Rainfall Data. The rainfall record for this period at the places where the wheats were grown is given in Table VII. This record indicates a dry period before the rains started on May 31. For most stations the interval between the date of heading and date of harvest was over one month. In most cases no rain occurred immediately after harvesting and hence the effects observed were due to the rain which fell while the wheat was standing in the fields. The rains which fell at Hutchinson on the two days after harvesting had no effect, since the wheat was combined.

Results. The data on test weight, flour yield, and ash, given in Table VIII, were obtained from a project underway to study the influence of environment on the quality of wheat varieties. One weakness in the data of Table VIII is that it is not known what figures would have been obtained in the absence of these rains. Comparisons can therefore be made only with what is generally obtained on unweathered wheat. On this basis the flour yields given in Table VIII are as high from wheats exposed to these rains as would be obtained from wheats not so exposed. The ash figures are also about what would be expected from wheat ripened under drier conditions.

TABLE VIII

Test Weight, Flour Yield, and Ash Values for Wheat Samples Grown at Various Locations in Kansas

Place grown	Rain	Test weight			Flour yield and flour ash					
		Black-hull	Chief-kan	Ten-marq	Blackhull		Chiefkan		Tenmarq	
					Yield	Ash	Yield	Ash	Yield	Ash
	inches	*lb*	*lb*	*lb*	%	%	%	%	%	%
Manhattan	14.11	55.2	57.0	54.2	71.4	0.39	73.4	0.44	74.2	0.43
Thayer	13.32	56.5	—	57.9	68.8	0.43	—	—	72.8	0.42
Hays	11.45	57.5	60.6	56.2	69.0	0.42	73.0	0.43	71.8	0.42
Hutchinson	10.92	59.6	60.5	58.4	72.0	0.40	71.3	0.42	71.8	0.42
Columbus	7.84	54.5	—	55.2	67.3	0.42	—	—	73.5	0.43
Kingman	6.74	57.9	58.5	55.8	70.5	0.43	72.0	0.43	73.0	0.42
Tribune	6.27	—	61.0	58.9	—	—	72.5	0.43	72.0	0.43
Meade	5.91	59.2	59.5	56.9	70.8	0.44	71.6	0.46	72.0	0.44
Colby	4.19	58.3	61.6	57.9	71.0	0.43	73.3	0.42	73.0	0.42
Garden City	3.50	—	59.8	55.7	—	—	69.0	0.51	71.4	0.46
Dodge City	2.98	57.4	60.3	52.5	71.0	0.43	72.8	0.47	68.5	0.46

The figures for internal texture given in Table IX show that the Blackhull samples were the least vitreous or most mealy, with the Tenmarq samples intermediate. Thus, the samples grown in various parts of the state show substantially the same effects from the rains

TABLE IX

INTERNAL TEXTURE VALUES FOR WHEAT SAMPLES GROWN AT VARIOUS LOCATIONS IN KANSAS

Place grown	Rain	Blackhull			Chiefkan			Tenmarq		
		Vit.	Semi vit.	Mealy	Vit.	Semi vit.	Mealy	Vit.	Semi vit.	Mealy
	inches	%	%	%	%	%	%	%	%	%
Manhattan	14.11	11	44	45	71	23	6	6	53	41
Thayer	13.32	53	31	16	—	—	—	74	15	11
Hays	11.45	53	38	9	96	4	0	69	25	6
Hutchinson	10.92	49	41	10	99	1	0	90	8	2
Columbus	7.84	47	25	28	—	—	—	64	14	22
Kingman	6.74	22	57	21	70	25	5	52	31	17
Tribune	6.27	—	—	—	96	0	4	93	7	0
Meade	5.91	58	31	11	90	7	3	72	19	9
Colby	4.19	36	50	14	97	2	1	88	10	2
Garden City	3.50	—	—	—	89	10	1	64	33	3
Dodge City	2.98	69	26	5	90	7	3	72	19	9

as those from Manhattan. There seems to be no consistent relationship between the size of the rains and the effects, as shown by the results from places of largest, medium, and smallest rainfall. This is indicated by the results from the three groups in Tables VIII and IX. That is, frequent small rains will decrease the test weight and change the internal texture as well as the larger rains.

The protein and loaf volumes given in Table X, also obtained from the project on influence of environment, show that Chiefkan was the

TABLE X

PROTEIN AND LOAF VOLUME OF FLOURS MILLED FROM WHEATS GROWN AT VARIOUS LOCATIONS IN KANSAS

Place grown	Blackhull		Chiefkan		Tenmarq	
	Protein [1]	Loaf vol.	Protein [1]	Loaf vol.	Protein [1]	Loaf vol.
	%	*cc*	%	*cc*	%	*cc*
Manhattan	12.4	843	13.6	783	12.8	943
Thayer	12.9	847	—	—	11.4	770
Hays	16.4	900	16.2	825	16.0	1142
Hutchinson	13.0	841	12.7	740	12.4	797
Columbus	10.5	702	12.8	—	9.8	683
Kingman	13.1	837	14.8	743	13.2	915
Tribune	—	—	16.1	733	14.7	958
Meade	15.7	927	—	888	14.8	1000
Colby	14.6	912	13.3	847	13.3	900
Garden City	—	—	18.3	915	18.0	1315
Dodge City	15.1	958	15.1	898	15.6	1207

[1] Protein content ($N \times 5.7$) is expressed on the "as is" moisture basis.

poorest in baking value although it was the best from the grain-grading standpoint, as shown by the test weight and texture values given in Tables VIII and IX. Tenmarq gave the largest loaf volumes except when it was lower in protein. All the loaves from Blackhull were larger than the comparable samples from Chiefkan.

The figures for protein in Table X show also that high protein wheat is not inconsistent with a comparatively large rainfall during heading.

Summary and Discussion

The data which have been presented on the effects of frequent rains accompanied by storms during the heading and ripening period indicate the same general effects as were obtained from the smaller rains reported previously (Swanson, 1943a). The larger rains caused greater mechanical losses in the field, but these were not reflected in the quality. The results of soaking wheat in the heads and in the grain indicate that the glumes afford some protection against the entrance of water into the kernels by absorption, such as takes place in soaking grain. The entrance of water into the interior of the kernels seems to be by molecular diffusion as was discussed in the previous report (Swanson, 1943a). Thus a small rain at night, or one followed by cool, cloudy weather may have as much effect as a larger rain followed by sunshine.

The data presented in this and the previous papers (Swanson, 1943, 1943b) show that lowering of test weights due to rains on wheat in the field, does not decrease the flour yield, increase the ash, nor lower the baking value. Sprouting was not observed in these samples. The size of the rains, except for mechanical losses, is of less consequence than the duration of the period of wetness which allows the molecular diffusion of water into the kernels. The causes of the lowering of test weight, decrease in the vitreous condition, and increase in the mealy texture are the loosening of the bran layer and the disturbing of the structure and arrangements of the material in the interior of the kernels as has been explained in previous papers (Swanson, 1941, 1943a).

These studies indicate that too much emphasis is placed on test weight in grading weathered wheat. This is of greater significance now because combining has become the prevailing method of harvesting wheat. The present grain grades were established when the grain binder was the common harvesting implement. Cutting could then be started when the grain had as much as 30 to 35% moisture.

Wetting and drying the grain before it has become hard has comparatively little effect on milling qualities. When the combine is used, the moisture should be about 13% and preferably less. Frequent rains on such wheat will lower the test weight with consequent de-

pression in grade. That the farmer who has weathered wheat is unduly penalized because major emphasis is placed on test weight in grading is clearly shown by the results of these investigations. These studies have indicated that the lowered wheat grades, due to rain, are not correspondingly reflected in decreased flour yields nor in baking values.

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FACTORS WHICH INFLUENCE THE PHYSICAL PROPERTIES OF DOUGH. VI. EFFECT OF CYSTEINE AND SOME OTHER SUBSTANCES ON MIXOGRAM PATTERNS [1]

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In a previous publication (Swanson, 1940, 1940a), it was shown that the pattern of curves or mixograms (Swanson and Johnson, 1943) was markedly influenced by dough rest periods as well as by the presence of cysteine monohydrochloride, the action of cysteine [2] being very vigorous. The main effect of cysteine was a shortening of the developing and the weakening periods as shown by steeper ascending and descending slopes of the mixogram patterns. Swanson and Dines (1939) found that the addition of cysteine in the preparation of the doughball for

[1] Contribution No. 100, Department of Milling Industry, and No. 281, Department of Chemistry.
[2] For brevity, cysteine monochloride will be referred to as cysteine throughout this paper.

the wheat-meal time test notably shortened the time required for disintegration to start and this was in direct proportion to the amount of cysteine added. In this respect the action of cysteine was similar to that of proteases (Swanson, 1939).

The effects of glutathione and cysteine on farinograms and baking were included in the studies by Sullivan, Howe, and Schmalz (1936). Swanson and Andrews (1942, 1943) have shown that the presence of certain surface-active or wetting agents markedly lengthens the developing and weakening periods. The wetting agents acted mostly, if not altogether, on the gluten materials as indicated by the characteristics of patterns obtained from mixtures of finely ground dry gluten and wheat starch.

The three main objects of the present investigation were to compare the influence on mixogram patterns of (1) cysteine when used alone and in several combinations with the wetting agent, Aerosol OT (sodium dioctylsulfosuccinate), and with sodium chloride; (2) cysteine and hydrogen sulfide, ethyl mercaptan, and isopropyl mercaptan; and (3) cysteine and cystine. The effects of these agents were judged by the variations they produced in the mixogram patterns.

Materials and Methods

Tenmarq flour of 13.3% protein was used for all the mixograms. This flour had a fairly long period of mixing, and hence was suitable for use with substances which shorten this time. For each mixogram, 35 g of flour and 22 ml of water or solution of the various substances under investigation were employed. In placing the substances in the mixing bowl the most active ingredients were added last so that their time of contact with the flour would be nearly the same as the mixing period. The amounts of the various agents are given in the legends to the figures presenting the mixograms.

Comparative baking tests were also made with Tenmarq and Chiefkan flour to determine the effects of Aerosol OT and cysteine on mixing time and loaf volume.

Results

Effects on Mixogram Patterns

Cysteine in Combination with Aerosol OT. The opposite effects of cysteine and Aerosol OT are shown in the upper two lines of mixograms in Figure 1. Increasing amounts of cysteine shortened the time to reach minimum mobility from 3.5 minutes with water alone to 1.5 minutes with 10 mg of cysteine. The increasing amounts of Aerosol OT lengthened the time to about 7 minutes. Since these two substances influence the patterns in opposite directions, it would be ex-

pected that they would overcome each other's effects. The mixograms obtained with these two substances in conjunction (lines 3, 4, and 5) show the various patterns possible. As cysteine was increased, more and more Aerosol OT was required to overcome the effects of the cysteine, and when 9 mg were used, even the larger amounts of Aerosol OT did not suffice.

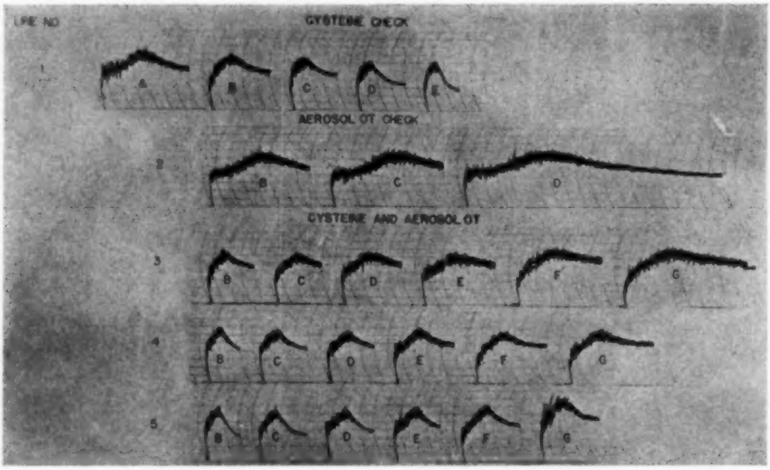

Fig. 1. Effect of cysteine, Aerosol OT, and combinations thereof on the mixogram pattern.

Line	Chemical added	A	B	C	D	E	F	G
		mg	*mg*	*mg*	*mg*	*mg*	*mg*	*mg*
1	Cysteine	0	2	4	6	10		
2	Aerosol OT		20	40	60			
3[1]	Cysteine+Aerosol OT		3+0	3+20	3+40	3+60	3+80	3+100
4[1]			6+0	6+20	6+40	6+60	6+80	6+100
5[1]			9+0	9+20	9+40	9+60	9+80	9+100

[1] 1st figure in each column represents cysteine, 2nd figure represents Aerosol OT.

Cysteine Alone and in Combination with Sodium Chloride. The presence of various salts in the wash water for gluten was shown by Dill and Alsberg (1924) to influence notably the yield of gluten. Sharp and Gortner (1924) found that as dough fermentation by yeast progressed, it became more and more difficult to wash gluten from dough with distilled water, but with 1% sodium chloride solution as much gluten was obtained after 8 hours fermentation as from unfermented dough.

The stiffening effect of sodium chloride on dough is shown in the upper line of mixograms in Figure 2; the more salt that is used, the

greater the effect. When sodium chloride was combined with cysteine (lines 2, 3, and 4) the stiffening effects were still apparent, but unlike Aerosol OT (Fig. 1) there were no increases in the time required to reach minimum mobility. The patterns in Figures 1 and 2 show that although the effects of both sodium chloride and Aerosol OT persist in the presence of cysteine, their actions are different. This stiffening effect is apparently due to a change in the character of the water caused

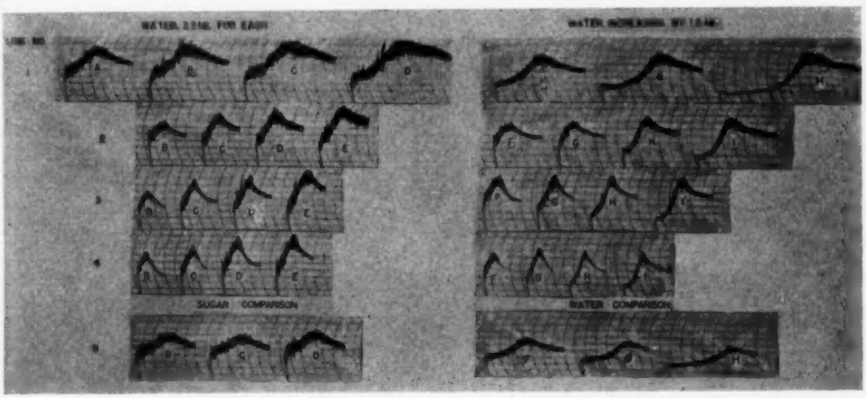

Fig. 2. Effect of cysteine in combination with sodium chloride on the mixogram pattern. Mixograms were made at constant absorption and also at augmented absorptions in proportion to the quantity of sodium chloride added. For comparative purposes, mixograms are shown for flour-water and flour-water-sucrose mixtures.

Line	Volume of solution constant					Water increment 1.5 ml per 0.35 g NaCl			
	A	B	C	D	E	F	G	H	I
1[1]	0+0	0+0.35	0+0.70	0+1.05		0+0.35	0+0.70	0+1.05	
2[1]		3+0.0	3+0.35	3+0.70	3+1.05	3+0.0	3+0.35	3+0.70	3+1.05
3[1]		6+0.0	6+0.35	6+0.70	6+1.05	6+0.0	6+0.35	6+0.70	6+1.05
4[1]		9+0.0	9+0.35	9+0.70	9+1.05	9+0.0	9+0.35	9+0.70	9+1.05
5[2]	0.70	1.40	2.1				23.5	25	26.5

[1] In lines 1 to 4 inclusive, first figure in each column represents mg cysteine, second figure represents g NaCl.
[2] Figures in Columns A, B, C represent grams sucrose. Figures in columns G, H, I represent ml water alone.

by a possible appropriation of the water by the ions from the sodium chloride. This is indicated in the patterns to the right. The addition of 1.5 ml of water for each 0.35 g of sodium chloride does not reduce the pattern heights, but with the slacker dough more and more time is needed to reach the peak. This stiffening or increase in plasticity appears to result from the binding of the water molecules to the ions from sodium chloride. With sodium chloride the plasticity is much decreased as can be seen by the decreased heights of the last three

patterns on line 5. In contrast to sodium chloride, sucrose has apparently little effect on plasticity.

Sulfhydryl Compounds. The powerful action of cysteine upon dough would seem to indicate that the cysteine molecule has a structural group peculiarly active in effecting changes in the protein. Of the various groups in the cysteine molecule the -SH group would seem to be of the most importance in causing these changes. Three other substances, hydrogen sulfide, ethyl mercaptan, and isopropyl mercaptan, which contain such groups, were used in this study.

The effects of the presence of -SH groups on flour and dough properties have been studied by Balls and Hale (1936, 1936a) and by Sullivan, Howe, and Schmalz (1936). Ziegler (1940, 1940a, 1940b), as well as others, has included glutathione in his studies of the changes which take place in dough.

The mixograms in line 1 of Figure 3 indicate that cysteine acts principally on the gluten proteins. Gluten was prepared from the Tenmarq flour used for the previous mixograms and blended with wheat starch, employing the method of Swanson and Andrews (1943). The protein content of this gluten was 77.5% on the air-dry basis, which indicates that considerable amounts of nongluten substances were present. Their influence was probably not important, as preliminary trials indicated that 8 g of air-dry gluten, finely ground, 27 g commercial wheat starch,[3] and 25 ml of water would give mixograms similar to those obtained from flour alone. This is shown by A in line 1. The effects of increasing amounts of cysteine are shown in the other mixograms in this line.

Cysteine is related to cystine by the union of two cysteine molecules through a disulfide linkage. Increasing amounts of a 5% solution of cystine in dilute HCl (line 2) do not show effects comparable to those of cysteine. The mixograms in line 3 were made with increasing amounts of 1.0*N* HCl and the patterns obtained show a strong similarity to those in line 2. This indicates that the small effect from cystine was due to the HCl used as a solvent.

The mixogram patterns obtained with saturated aqueous solutions of hydrogen sulfide, ethyl mercaptan, and isopropyl mercaptan shown in Figure 3 strongly resemble those obtained with cysteine (Figs. 1 and 2). These results indicate that the -SH group, which is common to these different substances, is the main causative agent in influencing the mixogram patterns. A few trials made with $Na_2S \cdot 9H_2O$ gave similar patterns.

[3] This starch was furnished by the Huron Milling Co., Huron, Michigan.

Effects on Baking

Only a few trials have been made on the effects of a wetting agent and cysteine on baking results. The loaves in Figure 4 which were baked by a rich formula containing dry milk and shortening show some possibilities.[4] The addition of 200 mg of Aerosol OT per loaf (100 g

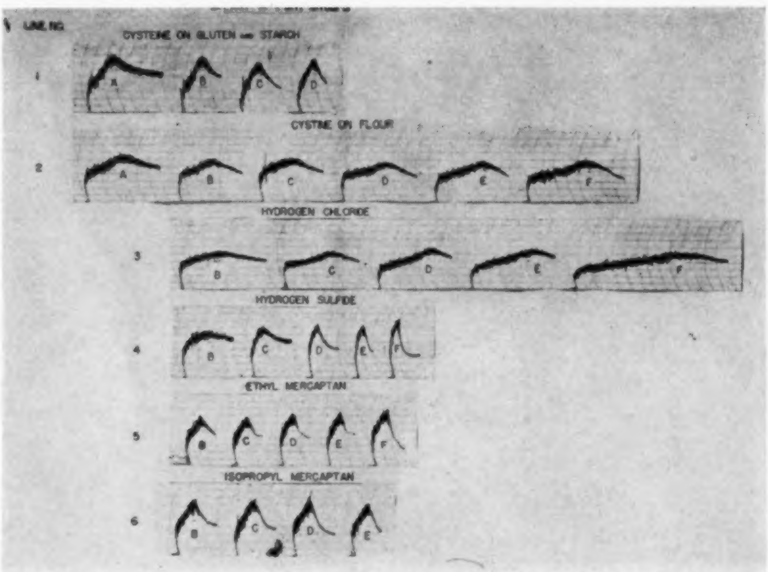

Fig. 3. Effect of hydrogen chloride and various sulfur-containing compounds on the mixogram pattern.

Line	Chemical added	A	B	C	D	E	F
1	Cysteine mg [1]	0	4	8	12		
2	Cystine ml 5% sol.	0	1	2	3	4	6
3	Hydrogen chloride ml 1.0N sol.		1	2	3	4	6
4	Hydrogen sulfide ml sat. sol.		1	3	6	10	15
5	Ethyl mercaptan ml sat. sol.		1	2	3	4	5
6	Isopropyl mercaptan ml sat. sol.		3	5	7	10	

[1] The cysteine was used on mixtures of dried, finely ground gluten and starch.

flour) approximately doubled the mixing time for doughs prepared from both Tenmarq and Chiefkan flours, whereas the addition of 8 mg of cysteine per 100 g of Tenmarq flour reduced the mixing time to 2.0 minutes, as compared with 3.4 minutes for the control.

The loaves containing Aerosol OT and cysteine are equal to the checks. Thus, decreasing the mixing time of Tenmarq with cysteine

[4] The authors are indebted to Mr. John A. Johnson, Assistant Baking Technologist, for making the baking tests.

and increasing that of Chiefkan with Aerosol OT resulted in as good volume and texture as when these substances were not present. This indicates the possibilities of altering the mixing time and still obtaining good bread. Use of cysteine and Aerosol OT in baking is being investigated further.

Fig. 4. Typical loaves, showing the effect of cysteine and Aerosol OT on baking properties of flour.

Loaf No.	Treatment	Mixing time	Loaf volume
		min	*cc*
1	Check, Tenmarq (13.3% protein)	3.4	930
2	200 mg Aerosol OT	6.4	928
3	Check, Chiefkan (13.0% protein)	1.7	728
4	200 mg Aerosol OT	3.5	778
5	8 mg cysteine	2.0	933

Discussion

In evaluating the magnitude of the opposite influence of Aerosol OT and cysteine on mixogram patterns, it becomes apparent that the effects are proportional to the molecular concentration of these substances. The concentrations were adjusted to procure pen swings of comparable magnitude and similar shape. The amounts thus used, as indicated in the figure legends, seem to bear little relationship until calculated on the molecular basis. As the molecular weight of Aerosol OT is about 3.7 times that of cysteine, it would require approximately 3.7 times the

weight of Aerosol OT to produce the equivalent effect of cysteine if each were of equal potency in their action upon dough.

Sodium chloride increases the dough stiffness, as shown by the increased mixogram heights, but the stiffening effects were naturally much less when the water was increased by 1.5 ml for each 0.35 g of sodium chloride used. Sodium chloride also tends to minimize the effects of cysteine. That the action of cysteine results from the -SH group is indicated by the similar effects obtained with hydrogen sulfide, ethyl mercaptan, and isopropyl mercaptan, all of which contain -SH groups.

Certain selected mercaptan patterns could be exactly superimposed upon certain cysteine patterns. The same was true for hydrogen sulfide. Since no alteration in curve pattern was obtained with the addition of cystine, it seems that the -SH group, rather than the $-NH_2$ or -COOH group, is the one which is active in altering dough characteristics.

The main effects of cysteine and of the substances which had similar results on the mixogram patterns are shown in steeper slopes; the heights are not greatly decreased. This indicates that the water makes contact with the gluten more quickly. But as soon as the dough has attained its maximum stiffness, weakening or slackening starts and proceeds at a much faster rate than for a corresponding flour-water dough. The behavior on the downslope suggests that the amounts of free water in the dough begin to increase as soon as the peak is passed, and this process gradually continues, as shown by the progressive slackening of the dough. The addition of cysteine to Tenmarq produced a mixogram similar to that for Chiefkan flour-water dough.

The counteracting effects of sodium chloride when used with cysteine may be due to an association of the polar water molecules with the ions from sodium chloride, producing ion hydration (Gortner, 1938). The more water molecules which are thus associated, the fewer there are which are free in the water films to influence dough mobility. The degree of mobility of the dough results from the varying freedom of the water molecules in the layers of water which cover the gluten strands or the starch granules. The thicker these layers, as related to the water absorption, the greater the mobility of the dough. Workers in this laboratory have found that increases in absorption will decrease the heights of mixograms but increase the time required to reach minimum mobility. This increased mobility has also been observed when sodium chloride is used and the amount of water is increased.

The stiffening action of sodium chloride upon dough seems to indicate a salting-out effect upon the protein. As the proteins become

salted out, they tend to lose their own water of hydration to the stronger hydration forces around the ions from the sodium chloride (Debye, 1927). Thus the protein strands seem to behave as though separated by water layers which have less freedom of molecular movement and the protein itself seems to become less highly hydrated and to stiffen.

Summary

Mixogram patterns can be markedly changed by the use of certain substances. Cysteine decreases the time to reach minimum mobility, while Aerosol OT (sodium dioctylsulfosuccinate) produces opposite results. Thus, by a suitable choice of the amounts of these reagents, the time factor in the mixogram pattern may be made longer or shorter.

Sodium chloride has a stiffening effect on the dough, as indicated by increased heights of the mixograms, and this action persists in the presence of cysteine. This stiffening effect was minimized by increasing the water by 1.5 ml for each 0.35 g of sodium chloride added.

Other substances containing -SH groups, such as hydrogen sulfide, ethyl mercaptan, and isopropyl mercaptan, influenced the mixogram pattern in a manner similar to cysteine. This indicates that the -SH group in cysteine is the main cause of its action on dough.

The few results obtained in baking indicated that the presence of cysteine or Aerosol OT, in the amounts used, had no deleterious effects on loaf volume or texture.

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EFFECT OF HYDROCYANIC ACID ON THE BAKING QUALITY OF FLOUR

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(Received for publication November 4, 1943)

The extensive use of hydrocyanic acid as a fumigant for flour makes the question of its retention by that material one of great importance. Marchadier *et al* (1921) reported finding 82 ppm of hydrocyanic acid in flour and stated that food prepared from this flour tasted of cherry laurel.

Griffin *et al* (1923), using sodium cyanide and the pot method of generation in dosages of from 1 to 6 oz per 100 cu ft of space, found up to 200 ppm of hydrocyanic acid in flour immediately after fumigation. However, after 4 days' storage in a large, well-ventilated room at 70°F practically all the hydrocyanic acid had left the flour.

Moucka (1936) fumigated wheat and rye flour for 48 hours with 1% of hydrocyanic acid gas by volume. Immediately after treatment the wheat flour showed 3–4 ppm, the rye flour 7–9 ppm. After 24 hours' aeration both were completely free from hydrocyanic acid.

Dean and Swanson (1911) published on the effect of common mill fumigants on the baking qualities of wheat flour. They fumigated three grades of hard winter wheat flour and four grades of soft winter wheat flour with hydrocyanic acid generated from potassium cyanide at the rate of 1 lb per 1000 cu ft of space for 12 hours at 90°F. Baking

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[2] Head, Department of Milling Industry, Kansas Agricultural Experiment Station. Contribution No. 106.

tests were made immediately after fumigation, repeated 2 or 3 days later, and again repeated after intervals of 30 and 60 days. They conclude, "An examination of the tables and plates will show that the effects of fumigation are so small as to be negligible. It is only in the careful measurements employed in the test that any difference between the fumigated and unfumigated flour is apparent at all. The only notable difference appears in the maximum volume of the dough in the test made immediately after the fumigation, but not after 30 days. The finished loaf shows no deleterious effect from fumigation in any of the tests."

Very little work has been reported in the literature on the effect of hydrocyanic acid on the baking quality of flour. The results reported at this time are limited but it appeared that they would be of interest to handlers of food products. Increased emphasis is now placed upon freedom of such products from insect life.

Experimental

The first experiments were conducted with a prepared ginger-cake flour. This flour was packed in 12-oz cartons and wrapped in cellophane. The packaged flour was fumigated in a vacuum vault of 2550-cu ft capacity for 18 hours with a total dosage of 5 lb of liquid hydrocyanic acid and a vacuum of 26 inches.

Immediately after the vault was unloaded a package was emptied into a glass jar and sealed. As soon as possible it was analyzed for the hydrocyanic acid retained. The hydrocyanic acid was determined by distilling it from a 20-g sample of flour to which 1 g of tartartic acid had been added. The HCN in the distillate was titrated with silver nitrate by the well-known Liebig method. Other samples were taken at 24-hour intervals for analysis from packages stored in the warehouse. Figure 1 presents the data for these analyses. All results are based on two closely agreeing analyses. The high initial concentration and the rapid loss of hydrocyanic acid are very interesting. The entire package at the highest concentration would contain 0.05 g of hydrocyanic acid. According to the figure given by Gettler and Baine (1938) as to the minimum lethal dosage (MLD) of hydrocyanic acid this would be about one-half the MLD for an adult.

Samples of the prepared ginger-cake flour were taken from the carton sealed immediately after the fumigation and baked. The cake was analyzed for hydrocyanic acid, but none was found. The rest of the cake was eaten by the laboratory staff with no ill effects.

Reports have reached the writers lately of difficulty experienced by bakers using recently fumigated flour. In order to determine what interval of time must elapse between fumigation and the complete loss

of hydrocyanic acid the following work was undertaken: A 48-lb bag of patent flour was fumigated for 24 hours in a vault at normal atmospheric pressure with a dosage of 2.25 oz of liquid hydrocyanic acid per 1000 cu ft of space. This low dosage was adopted so that the single bag used would absorb approximately the same quantity as would one of a warehouseful during a normal fumigation. Immediately on its

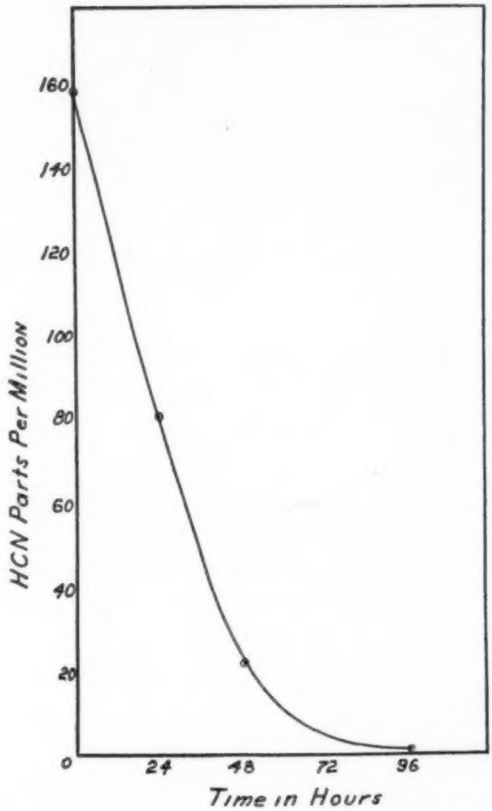


Fig. 1. Loss of hydrogen cyanide from fumigated prepared cake flour.

removal from the vault the bag of flour was sampled, and other samples were taken at 24-hour intervals thereafter. The results are represented in Figure 2. The samples were baked using 3% shortening superimposed upon the lean formula and procedure described in detail by Johnson, Swanson, and Bayfield (1943). Results are presented in Table I.

The volume of the loaf may be seen to have been definitely depressed in the sample taken immediately after fumigation, amounting

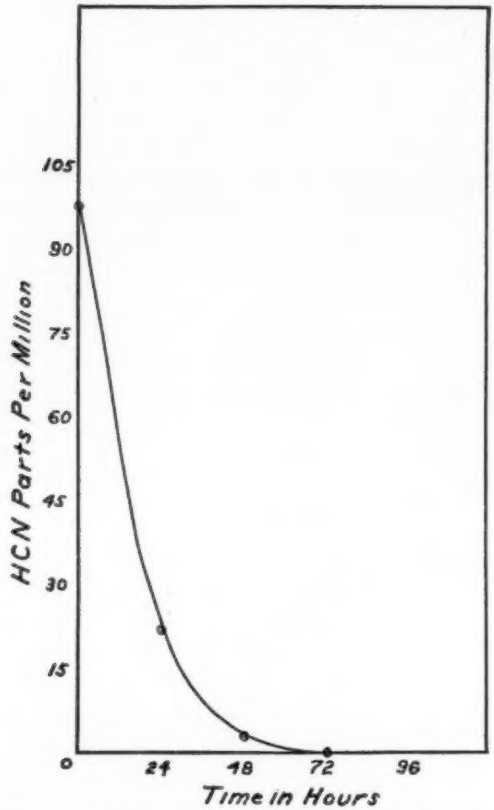


Fig. 2. Loss of hydrogen cyanide from fumigated bread flour.

to about 11% of the check. After 24 hours the volume was still somewhat depressed. The differences shown by the 48- and 72-hour samples are not significant. No odor of hydrocyanic acid could be

TABLE I

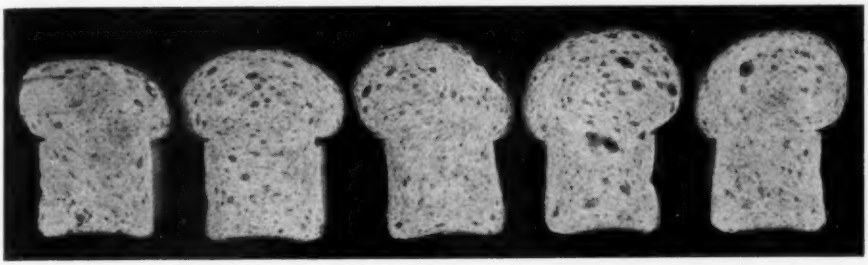
BAKING RESULTS AFTER FUMIGATION WITH HYDROGEN CYANIDE [1]

Aeration	Mixing time	Loaf volume	Crumb color [2]	Crumb grain and texture [3]
hours	*min*	*cc*		
0	2.0	888	82 cy	80-o
24	2.5	945	85 cy	83-o
48	2.7	990	87 cw	87-o
72	3.3	1015	87 cw	85-o
Check	3.5	1000	87 cw	85-o

[1] Flour protein 12% (15% moisture basis), moisture 10.9%, absorption 68%. Results are averages of three fumigations.
[2] Cy = creamy yellow; cw = creamy white.
[3] o = open grain.

detected in the baked loaves. Since this odor test is very sensitive, no analysis of the baked loaves was deemed necessary.

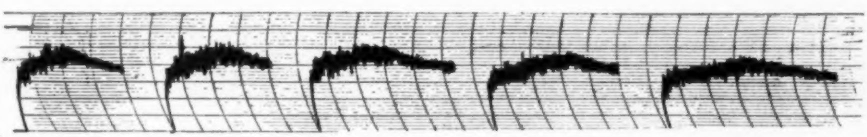
The difference in volume of the baked loaves, as shown in Figure 3, is readily observable. The consistency of the dough made from the



Immediately after fumigation | 24 hours later | 48 hours later | 72 hours later | Check

Fig. 3. Loaves of bread baked from fumigated flour at various intervals after fumigation with hydrogen cyanide.

different flours is shown in the "mixograms" presented in Figure 4. The nature of these recordings is completely described by Swanson and Johnson (1943). Briefly, they are graphs or curves made by a recording dough mixer known as a mixograph. Each graph constitutes



Immediately after fumigation | 24 hours later | 48 hours later | 72 hours later | Check

Fig. 4. Mixograms of fumigated flour, at various intervals after fumigation with hydrogen cyanide.

a record of the behavior of a dough during mixing and development by mechanical action. This record includes the rate of dough development, the maximum stiffness or resistance at complete mixing or minimum mobility, the tolerance or sensitiveness to mixing, and the rate of break-down or increase in mobility. Each vertical line on the mixograms represents an interval of approximately 1 minute. It may be seen from these curves that the effect of the hydrocyanic acid is directly on the flour, since they were obtained on the flour and water alone. These curves show a reduction in mixing time and a tendency toward increased slackness in the dough due to absorbed fumigant.

This work indicates that a baker about to have his warehouse fumigated should hold out enough unfumigated flour to last him for 3 days' baking to avoid trouble from the absorbed fumigant. It is likely that the HCN residue would not be lost so rapidly from a stack of bagged

flour as from a single sack as used in these tests. Therefore, fumigated flour should be thoroughly aerated before it is used in baking.

Summary

Hydrocyanic acid when used as a flour fumigant produces an appreciable and detrimental influence upon the bread baking quality. This effect is not apparent after thorough aeration of the flour.

Ginger cake from freshly fumigated flour was free of fumigant.

Acknowledgment

The authors are indebted to John A. Johnson for making the baking tests.

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NICOTINIC ACID IN PRODUCTS OF COMMERCIAL RICE MILLING AND IN RICE VARIETIES [1]

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(Received for publication January 6, 1944)

In previous communications (Kik, 1943; Kik and Van Landingham, 1943) studies were reported on thiamine and riboflavin in products of commercial rice milling and in rice varieties. This investigation deals with the nicotinic acid content of these products. In addition, samples of parboiled and undermilled rice have been tested.

The nicotinic acid content of wild rice has been reported by Nelson and Palmer (1942), and Williams, Knox, and Fieger (1943) recently

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[2] Resigned.

published a study of the vitamin B-complex factors in rice and its milled products.

The essential steps in milling, description of the main products and by-products, and the methods employed in obtaining representative samples were described in our previous papers. Nicotinic acid was determined by the colorimetric method of Melnick (1942); the analyses were made in duplicate, employing 1.0 g samples of the main products and 250 mg of the by-products. As a test of the suitability of the colorimetric method, the samples of whole brown rice employed in this study were submitted to another laboratory for microbiological

TABLE I

NICOTINIC ACID IN PRODUCTS OF COMMERCIAL RICE MILLING [1]

Products	Variety[2] and mill lot							
	Supreme Blue Rose		Early Prolific		Fortuna	Lady Wright	Improved Blue Rose[4]	Mean
	551	606	569	663	635	768	778	
	NICOTINIC ACID CONTENT (DRY MATTER BASIS)							
	µg/g	µg/g	µg/g	µg/g	µg/g	µg/g	µg/g	µg/g
Paddy or rough rice	49.8	49.8	48.5	47.7	46.4	55.4	47.3	49.2
From milling or bleaching process:								
Whole brown rice[5]	57.4	56.4	51.2	52.8	49.2	64.7	54.3	55.1
First break huller rice	25.0	25.2	22.8	21.2	22.3	24.6	24.9	23.7
Second break huller rice	24.5	23.3	21.6	20.9	21.4	20.2	24.8	22.4
Pearling cone rice	24.0	23.0	21.0	20.7	—[3]	—[3]	24.4	22.6
Brush rice	22.0	21.0	20.7	20.3	17.4	20.0	22.0	20.4
Finished, clean, polished rice:								
Head rice	18.9	17.5	20.0	19.7	15.6	19.5	18.0	18.4
Second head rice	17.2	16.5	19.5	19.1	15.5	18.9	15.6	17.7
Screenings	24.4	23.2	24.3	25.1	22.8	24.1	22.6	23.8
Brewers' rice	39.5	37.8	34.4	35.0	34.9	37.1	33.9	36.1
Rice by-products:								
Hulls	16.9	17.3	17.0	17.9	25.1	22.1	14.0	18.6
First break bran	320.0	338.0	340.8	358.2	315.1	303.2	349.0	332.0
Second break bran	306.0	316.0	259.5	265.1	283.1	262.8	311.7	286.3
Pearling cone polish	408.0	412.0	359.0	347.5	—[3]	—[3]	312.5	367.8
Brush polish	384.4	368.0	269.4	232.3	206.6	296.0	275.9	290.4

[1] Obtained from one mill through the courtesy of the Walton Mill, Inc., Stuttgart, Arkansas.
[2] All varieties are from fields which were not fertilized and were irrigated by well water.
[3] Pearling cones are not used in long grain varieties.
[4] Grown in Louisiana.
[5] The following values were obtained by John S. Andrews, Research Department, General Mills Inc., for the same samples of whole brown rice tested by the microbiological method: 57.1, 55.1, 53.3, 47.3, 53.5, 58.4, and 50.0. Average 53.5 µg/g. These values are in good agreement with those obtained by the colorimetric method.

assay.[3] For these assays the method described by Andrews, Boyd, and Gortner (1942) was used.

Results

Table I shows that on the dry basis rough rice contained an average of 49.2 μg/g of nicotinic acid, while brown rice contained 55.1 μg or slightly more than rough rice. An average of 53.5 μg was obtained for the same samples of whole brown rice, using the microbiological method, thus confirming the validity of the colorimetric method for the determination of nicotinic acid in these samples.

The decrease in mean percentage of nicotinic acid in converting brown rice to head rice amounted to 66.4%. These decreases for individual varieties ranged from 60.9% for Early Prolific to 70.0% for Lady Wright.

TABLE II

Nicotinic Acid Content of Milled Parboiled and Milled Nonparboiled Rice, Milled and Undermilled Rice

Variety	Nicotinic acid content (dry matter basis) for stated treatment			
	Milled, parboiled	Milled, not parboiled	Under-milled	Milled
	μg/g	μg/g	μg/g	μg/g
Nira[1]	49.0	—	—	—
Nira[1]	—	20.6	—	—
Caloro[2]	45.2	—	—	—
Caloro[2]	—	18.5	—	—
Indian[3]	45.0	—	—	—
Lady Wright[3]	—	—	26.2	—
Lady Wright[3]	—	—	—	19.5
Supreme Blue Rose[3]	—	—	26.6	—
Supreme Blue Rose[3]	—	—	—	18.9

[1] Obtained through the courtesy of C. R. Adair, Associate Agronomist, U. S. Department of Agriculture, Bureau of Plant Industry, Rice Branch Experiment Station, Stuttgart, Arkansas.
[2] Obtained through the courtesy of the Rice Growers Association of California, Sacramento, California.
[3] Obtained through the courtesy of the Arkansas Rice Growers Cooperative Association, Stuttgart, Arkansas.

The results of assays of a few samples of parboiled and undermilled rice are shown in Table II, and indicate that the nicotinic acid content of milled parboiled rice is considerably higher than that of milled non-parboiled rice. A sample of milled parboiled rice of the Nira variety contained 49.0 μg/g and a sample of milled parboiled California rice (Caloro variety) had 45.2 μg/g compared with an average of 18.5 μg/g for ordinary milled rices (Table I).

The nicotinic acid content of undermilled rice is higher than that of ordinary milled rice. An undermilled sample of Lady Wright con-

[3] Credit is due John S. Andrews, Research Department, General Mills, Inc., Minneapolis, Minnesota, for the Microbiological Assays of these samples.

tained 26.2 μg/g compared with 19.5 μg/g for a representative sample of milled rice.

Samples of 18 varieties of rough rice or paddy, obtained from the main rice-producing states, Arkansas, Louisiana, Texas, and California, were tested for their nicotinic acid content with the results shown in Table III.

TABLE III

NICOTINIC ACID CONTENT OF DIFFERENT VARIETIES OF PADDY OR ROUGH RICE GROWN IN ARKANSAS, LOUISIANA, TEXAS, AND CALIFORNIA (1941 HARVEST)

Variety	Nicotinic acid content (dry matter basis)			
	Arkansas[1]	Louisiana[2]	Texas[3]	California[4]
	μg/g	μg/g	μg/g	μg/g
Early Prolific	47.8	39.3	46.3	40.0
Caloro	46.0	49.6	52.6	—
Arkrose	55.7	48.6	—	—
Acadia	40.0	41.3	—	—
Prelude	41.5	43.2	—	—
Supreme Blue Rose	46.0	—	—	—
Improved Blue Rose	—	50.1	—	—
Blue Rose	—	—	46.5	—
Blue Rose	—	—	40.6	—
Blue Rose	—	—	44.2	—
Lady Wright	43.0	41.0	—	45.0
Nira	40.0	45.1	47.0	46.7
Arkansas-Fortuna	52.1	46.6	—	—
Zenith	53.3	56.8	52.0	48.6
Japan	—	—	52.4	—
Japan	—	—	46.8	—
Rexora	—	—	55.9	—
Rexora	—	—	54.7	—
Fortuna	—	—	59.0	—
Fortuna	—	—	51.1	—
Calady	—	—	—	40.7
Calady 40	—	—	—	43.4
Colusa	—	—	—	52.3
Average	46.5	46.1	49.9	45.2

[1] From Rice Branch Experiment Station, Stuttgart, Arkansas.
[2] From Rice Branch Experiment Station, Crowley, Louisiana.
[3] From Rice Grading Service, American Rice Growers' Cooperative Association, Beaumont, Texas.
[4] From Rice Experiment Station, Biggs, California.

The average nicotinic content of all varieties was 46.5 μg; the highest content (59.0) was found in a sample of Fortuna grown in California, and the lowest (39.3) was from a sample of Early Prolific grown in Louisiana. Small differences were found in the nicotinic acid content of varieties grown at the same location which indicates that varieties differ somewhat in their nicotinic acid content.

The apparent effect of locality on the nicotinic acid content is small. Early Prolific from four states showed similar nicotinic acid content; in Arkansas 47.8, Louisiana 39.3, Texas 46.3, and California 40.0.

Blue Rose grown in Texas in three different localities had a nicotinic acid content of 46.5, 40.6, and 44.2. Similar observations were made on the nicotinic acid content of the varieties Caloro, Lady Wright, Nira, and Zenith.

Summary

The nicotinic acid content of products of commercial rice milling and of rice varieties has been determined. The average for paddy or rough rice and for whole brown rice was 49.2 μg/g and 55.1 μg/g respectively.

Of the finished, clean products, the end product, head rice (sold for human consumption), contained an average of 18.4 μg and second head 17.7 μg/g of dry matter. An average of 66.4% of nicotinic acid was removed during the milling process.

Screenings and brewers' rice contained 23.8 and 36.1 μg nicotinic acid respectively.

Of the by-products, hulls contained 18.6 μg/g, bran from 262.8 to 358.2 μg/g, and rice polish 206.6 to 408.0 μg/g of nicotinic acid. Three samples of milled parboiled rice (prepared in three different localities) contained 49.0, 45.2, and 45.0 μg of nicotinic acid per gram of dry material. Two samples of undermilled rice contained 26.2 and 26.6 μg, compared with 19.5 μg and 18.9 μg/g in the milled rice.

The average nicotinic acid content of all varieties (rough rice) was 46.5 μg/g.

The nicotinic acid content of rice differed with variety and to a small extent with locality.

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